DFI was not significantly prognostic of survival following recurrence on either univariate or multivariate analysis in this study, as shown in table 4. There have been some controversies about the effect of DFI on OS after recurrence. Patients recurred with longer DFI had superior survival to those with shorter DFI in recurrent colon cancer [20]. On the contrary, the MST of metastatic patients pretreated with adjuvant chemotherapy was independent of DFI in recurrent breast cancer [21]. In this study, the numbers of patients with a DFI less than 1 year, from 1 to 2 years, from 2 to 3 years and more than 3 years were 43, 29, 9 and 8, respectively. It remains possible for DFI to become a prognostic factor if the number of patients with a long DFI increases.

Of note, the MST of 287 days following recurrence in patients initially treated with adjuvant S-1 after curative gastrectomy was similar to that of 7 months yielded by the subsequent chemotherapy to S-1 in advanced/recurrent gastric cancer [12, 14]. No matter who received the first-line chemotherapy of S-1 as an adjuvant one or not, the OS after the usage of S-1 might be the same in patients who had recurrent tumor left after S-1 administration.

Although we believe that this is the first report demonstrating that patients initially treated with S-1 adjuvant chemotherapy had significantly inferior survival following recurrence and poorer response to first-line chemotherapy, compared with those without any adjuvant treatment after curative gastrectomy, it should be noted that the present study is a retrospective small-sized analysis performed at a single center. The results shown here warrant further study to elucidate the effect of S-1 adjuvant chemotherapy in patients with recurrent gastric cancer and to investigate an optimal regimen for patients relapsed after adjuvant S-1, though a prospective randomized study seems infeasible because adjuvant S-1 has become the standard treatment for stage II-III gastric cancer patients in Japan.

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Comparison of efficacy of concomitant administration of mitiglinide with voglibose and double dose of mitiglinide in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: When monotherapy with an oral hypoglycemic agent (OHA) is not sufficiently effective for blood glucose control, combination therapy with OHA having different mechanisms of action might be indicated.

Materials and Methods: In the present study, we compared the efficacy of two options in type 2 diabetes mellitus patients whose blood glucose had not been well controlled with mitiglinide (30 mg/day) alone. A total of 20 patients were included in the study and divided into two groups: group A, in which mitiglinide was given concomitantly with the α-glucosidase inhibitor voglibose (0.6 mg/day); and group B, in which a double dose of mitiglinide was given (60 mg/day). Twelve weeks after changing the medication, HbA_{1c}, glycoalbumin and 1,5-anhydroglucitol (1,5-AG) were measured. In addition, at weeks 0 and 12, a meal tolerance test was carried out, and plasma glucose, insulin, glucagon, active glucagon-like peptide-1 (GLP-1) and total glucose-dependent insulinotropic polypeptide levels were measured.

Results: The plasma level of 1,5-AG improved in both groups at week 12. In group A, the plasma insulin level significantly decreased and the plasma active GLP-1 level significantly increased during the meal tolerance test at week 12; thus, bodyweight significantly decreased only in group A.

Conclusions: Our findings suggested that concomitant administration of mitiglinide with voglibose could achieve better glycemic control, particularly in the postprandial period, without bodyweight gain and might have beneficial effects in type 2 diabetic patients at risk of macrovascular complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00082.x, 2010)

KEY WORDS: α-Glucosidase inhibitor, Glucagon-like peptide-1, Mitiglinide

INTRODUCTION

Mitiglinide calcium hydrate (mitiglinide) is a potent, fast-acting, short-duration insulin secretagogue that promotes insulin secretion just after meals and thereby inhibits postprandial hyperglycemia. It is an oral hypoglycemic agent (OHA) that rarely induces hypoglycemia because of its short duration of action. α-Glucosidase inhibitors (α-GI) inhibit glucose absorption in the upper small intestine, improve postprandial hyperglycemia, and regulate delayed and excessive insulin secretion. These OHA are effective for both postprandial hyperglycemia and hyperinsulinemia. Furthermore, α-GI might have other beneficial effects on the secretion of incretins, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which have been reported to possess trophic effects on β-cells. In animal models, native GLP-1 stimulates β-cell proliferation and inhibits apoptosis, which might increase β-cell mass and function[1].

When monotherapy with an OHA is not sufficiently effective in blood glucose control, combination therapy with OHA having different mechanisms of action could be indicated. In case monotherapy with mitiglinide is not sufficient to treat postprandial hyperglycemia, one of the risk factors for macroangiopathy, it remains unclear whether it is better to increase the dose of mitiglinide or to use it concomitantly with an α-GI.

In the present study, we compared these two options in type 2 diabetes mellitus patients, whose plasma glucose levels had not been well controlled by dietary therapy and mitiglinide administration (30 mg/day t.i.d.).

METHODS

Subjects

The subjects included 20 outpatients with type 2 diabetes mellitus (age ≥ 20 years) whose plasma glucose levels had not been

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well controlled with dietary therapy and mitiglinide (10 mg/day t.i.d.) for at least 8 weeks before week 0. The protocol was approved by the Ethics Committee of Hyogo College of Medicine. Each participant gave written informed consent before the start of the study and fulfilled all the following inclusion criteria:

1 A HbA_{1c} level of 6.9–8.9% at week 0;
2 Not treated with OHA other than mitiglinide for 24 weeks (168 days) before week 0;
3 Not treated with insulin for over 8 weeks (56 days) before week 0.

The patient background characteristics (sex, age, duration of diabetes, body mass index [BMI], HbA_{1c} and levels of fasting plasma glucose, insulin, total cholesterol, low-density lipoprotein cholesterol, triglyceride, and blood pressure) at week 0 are summarized in Table 1.

Study Design

The study was carried out at the Division of Diabetes and Metabolism, Department of Internal Medicine, Hyogo College of Medicine and Watanabe Medical Clinic in Nishinomiya, Hyogo, Japan. The subjects were randomly allocated to two groups using a lottery system on an open trial basis: group A (concomitant voglibose group), in which mitiglinide was given concomitantly with voglibose; and group B (double mitiglinide group), in which a double dose of mitiglinide was given.

The study design is shown in Figure 1. During the observation period, 10 mg mitiglinide was given orally three times a day before meals (within 5 min before each meal). During the treatment period, 10 mg mitiglinide and 0.2 mg voglibose were given orally to subjects in group A three times a day before meals, whereas 20 mg mitiglinide was given orally to subjects in group B three times a day before meals. During the observation and treatment periods, neither agent was given after a meal or without a meal. The administration period was 24 weeks, including 12 weeks of observation and 12 weeks of treatment. Dietary therapy was started at least 8 weeks (56 days) before the start of the observation period, and continued without change throughout the study period. If exercise therapy had been used before the study, it was continued without change throughout the study period; however, subjects were not allowed to initiate exercise therapy during the study period. The levels of HbA_{1c}, glycoalbumin (GA) and 1,5-AG were measured at week 12. Considering the relationship between HbA_{1c} value measured by the traditional Japanese standard measurement method (JDS) and the National Glycohemoglobin Standardization Program (NGSP), the HbA_{1c} value (%) in the present study is

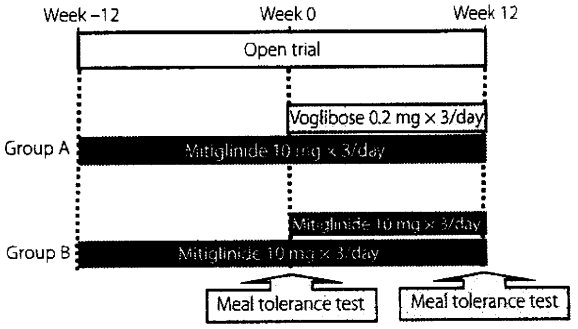

Figure 1 | Study design. Test meal (460 kcal, 56.5 g carbohydrate, 18 g protein, 18 g fat).

Table 1 | Patient characteristics

	All subjects	Group A (concomitant voglibose group)	Group B (double mitiglinide group)	*P*-value (A *vs* B)
No. subjects (male, female)	20 (12, 8)	10 (7, 3)	10 (5, 5)	–
Age (years)	59.9 ± 11.4	57.8 ± 10.9	62.0 ± 12.1	N.S.
Duration of diabetes (years)	9.8 ± 6.0	7.1 ± 3.6	12.4 ± 6.8	0.0460
BMI (kg/m^2)	24.6 ± 4.1	25.9 ± 2.6	23.2 ± 5.0	N.S.
HbA_{1c} (%)	7.8 ± 0.6	8.1 ± 0.6	7.4 ± 0.3	0.0130
Fasting plasma glucose level (mg/dL)	167.9 ± 27.3	179.5 ± 30.8	156.3 ± 18.0	0.0410
SBP (mmHg)	126.9 ± 5.6	125.8 ± 6.4	128.0 ± 4.9	N.S.
DBP (mmHg)	72.6 ± 2.7	73.4 ± 3.4	71.8 ± 1.5	N.S.
TC (mg/dL)	213.0 ± 29.6	210.5 ± 23.6	215.5 ± 35.8	N.S.
LDL-C (mg/dL)	128.7 ± 27.2	129.4 ± 16.9	128.0 ± 35.7	N.S.
TG (μU/mL)	123.0 ± 99.8	143.1 ± 122.2	102.9 ± 72.1	N.S.

BMI, body mass index; DBP, diastolic blood pressure; HbA_{1c}, hemoglobin A_{1c}; LDL-C, low-density lipoprotein cholesterol; N.S., not significant; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

shown as the NGSP equivalent value (%) calculated on the basis of the following formula[2]: HbA_{1c} (NGSP) (%) = HbA_{1c} (JDS) (%) + 0.4 (%). At weeks 0 and 12, a meal tolerance test was carried out using a test meal (JANEF E460F18: 460 kcal, 56.5 g carbohydrate, 18 g protein and 18 g fat). The levels of plasma glucose, insulin, glucagon, active GLP-1 and total GIP were measured at 0, 30, 60 and 120 min during the meal tolerance test.

The plasma level of active GLP-1 was measured by enzyme-linked immunosorbent assay (ELISA) using a GLP-1 Active ELISA Kit (Millipore Corporation, Billerica, MA, USA) according to the manufacturer's instructions. The plasma level of total GIP was also measured by ELISA using a Human GIP (total) ELISA kit (Millipore Corporation). Blood samples for measurement of GLP-1 and GIP were collected in EDTA tubes containing aprotinin and a dipeptidyl peptidase 4 inhibitor (10 μL/mL of blood; Millipore Corporation).

Bodyweight was measured every 4 weeks and the subjects were asked about the occurrence of hypoglycemia throughout the study period. The study was carried out from April 2008 to December 2009.

Statistical Analysis

Data are represented as mean ± standard deviation unless otherwise specified. For intergroup comparisons, the Wilcoxon signed-rank test was used. For multiple comparisons, two-way analysis of variance (ANOVA) was carried out. The results of ANOVA at each time-point of measurement were examined using the Wilcoxon signed-rank test. The area under the curve (AUC) was estimated using the trapezoid method.

RESULTS

Characteristics of Subjects

The characteristics of subjects in groups A and B at week 0 are presented in Table 1. The duration of diabetes was significantly shorter in group A than in group B. HbA_{1c} and fasting plasma glucose levels were significantly higher in group A than in group B (8.1 ± 0.6% *vs* 7.4 ± 0.3% and 179.5 ± 30.8 mg/dL *vs* 156.3 ± 18.0 mg/dL, respectively). BMI was also higher in group A than in group B, though not to a significant degree. Blood pressure and lipid profiles did not differ between the groups.

Changes in HbA_{1c}, GA and 1,5-AG Levels

In group A, 1,5-AG level had improved significantly at week 12 (3.5 ± 2.9 to 6.9 ± 6.6 μg/mL, $P = 0.0039$); GA and HbA_{1c} levels had also improved, though not to a significant degree. In group B, HbA_{1c}, GA and 1,5-AG levels were all improved significantly at week 12 (7.4 ± 0.3 to 7.2 ± 0.4%, $P = 0.0469$; 22.1 ± 2.7 to 20.5 ± 1.9%, $P = 0.0078$; and 4.1 ± 2.0 to 5.9 ± 3.6 μg/mL, $P = 0.0234$, respectively; Table 2).

Changes in Bodyweight and Hypoglycemic Events

In group A, bodyweight significantly decreased (71.9 ± 12.7 to 70.8 ± 12.6 kg, $P = 0.0039$), whereas in group B, there was no significant change (60.0 ± 14.0 to 59.8 ± 16.8 kg, $P = 0.5315$; Table 2). No symptoms of hypoglycemia were noted in either group throughout the study period.

Changes in Plasma Glucose and Insulin Levels in Meal Tolerance Tests at Weeks 0 and 12

Changes in plasma glucose levels after a meal are shown in Figure 2. In group A, plasma glucose levels 30 and 60 min after a meal significantly decreased at week 12 (245.7 ± 38.1 to 202.4 ± 37.5 mg/dL, $P = 0.0039$ and 273.0 ± 51.9 to 229.2 ± 39.2 mg/dL, $P = 0.0059$, respectively). The plasma glucose level peaked 60 min after a meal at week 0 and 120 min after a meal at week 12. In group B, up to 30 min after a meal, the mean plasma glucose level at week 12 remained almost the same as that at week 0. The mean plasma glucose level 120 min after a meal improved at week 12, though not to a significant degree.

Table 2 | Changes in parameters from week 0 to 12 in groups A and B

	Group A (concomitant voglibose group)		*P*-value (*vs* week 0)	Group B (double mitiglinide group)		*P* value (*vs* week 0)
	Week 0	Week 12		Week 0	Week 12	
HbA_{1c} (%)	8.1 ± 0.6	7.9 ± 0.8	N.S.	7.4 ± 0.3	7.2 ± 0.4	0.0469
GA (%)	22.3 ± 2.2	21.8 ± 3.4	N.S.	22.1 ± 2.7	20.5 ± 1.9	0.0078
1,5 AG (μg/mL)	3.5 ± 2.9	6.9 ± 6.6	0.0039	4.1 ± 2.0	5.9 ± 3.6	0.0234
Fasting plasma glucose (mg/dL)	179.5 ± 30.8	168.7 ± 27.6	N.S.	156.3 ± 18.0	150.5 ± 16.2	N.S.
Weight (kg)	71.9 ± 12.7	70.8 ± 12.6	0.0039	60.0 ± 14.0	59.8 ± 16.8	N.S.
Glucose AUC_{0-120} (mg h/dL)	30196.5 ± 5627.4	26044.5 ± 4394.3	0.0098	25363.5 ± 5443.6	24180.0 ± 5366.1	N.S.
Insulin AUC_{0-120} (μU h/mL)	3741.8 ± 2184.6	3229.8 ± 1551.8	N.S.	2878.0 ± 1840.5	3221.1 ± 2365.3	N.S.
GLP-1 AUC_{0-120} (pmol h/L)	648.9 ± 91.6	843.3 ± 336.9	0.0137	604.2 ± 58.8	664.5 ± 103.7	N.S.
GIP AUC_{0-120} (pg h/mL)	24151.1 ± 9506.3	22856.1 ± 10277.1	N.S.	24481.2 ± 8888.7	26751.1 ± 12145.2	N.S.
Glucagon AUC_{0-120} (pg h/mL)	10347.6 ± 2029.6	11090.4 ± 1948.1	N.S.	10373.0 ± 2590.2	9820.5 ± 2151.4	N.S.

1,5-AG, 1,5-anhydroglucitol; AUC, area under the curve; GA, glycoalbumin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; HbA_{1c}, hemoglobin A_{1c}; N.S., not significant.

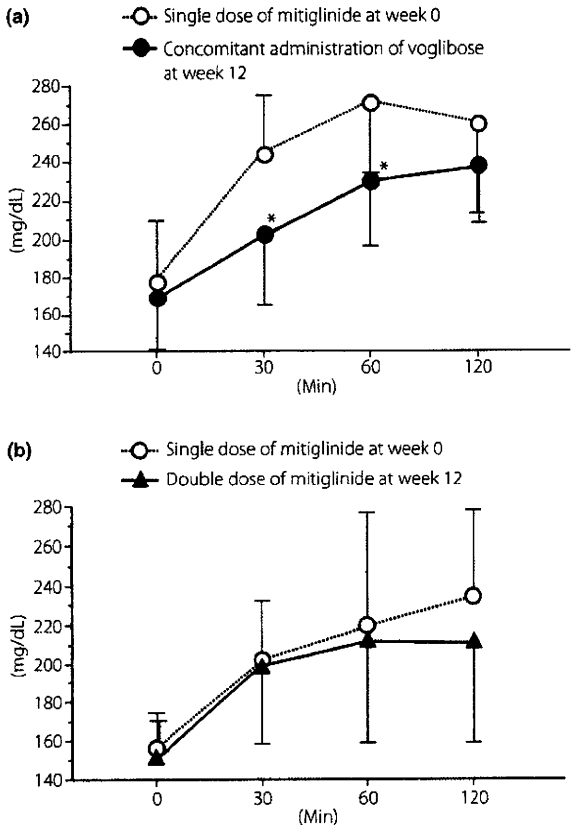

Figure 2 | Changes in plasma glucose level after meal tolerance test from week 0 to 12 in (a) group A and (b) group B. A significant decrease 30 and 60 min after a meal was observed at week 12 in group A. *$P < 0.05$ *vs* before addition of voglibose (Wilcoxon signed-rank test).

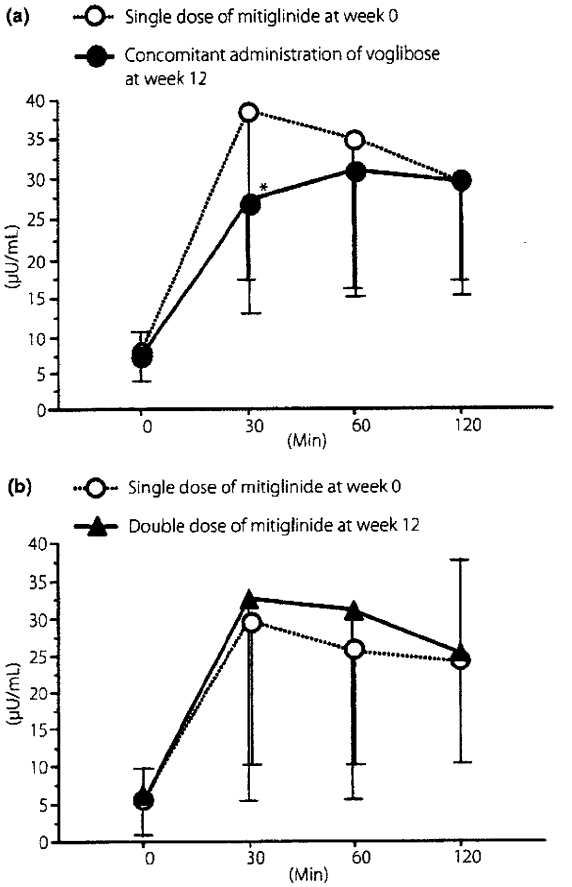

Figure 3 | Change in plasma insulin level after meal tolerance test from week 0 to 12 in (a) group A and (b) group B. A significant decrease 30 min after a meal was observed at week 12 in group A. *$P < 0.05$ *vs* before addition of voglibose (Wilcoxon signed-rank test).

The plasma glucose level peaked 120 min after a meal at week 0 and 60 min after a meal at week 12.

Changes in plasma insulin level and AUC are presented in Table 2 and Figure 3. In group A, the plasma insulin level 30 min after a meal significantly decreased at week 12 (38.5 ± 27.0 to 27.3 ± 10.4 μU/mL, $P = 0.0273$). AUC_{0-30} of the plasma insulin level significantly decreased (698.3 ± 227.1 to 521.8 ± 215.6 μU h/mL, $P = 0.0273$). AUC_{0-120} of the plasma insulin level throughout the meal tolerance test at week 12 in group A was less than that at week 0, though not to a significant degree (3741.8 ± 2184.6 to 3229.8 ± 1551.8 μU h/mL). The plasma insulin level peaked 30 min after a meal at week 0 and 60 min after a meal at week 12. In group B, the plasma insulin levels 30 and 60 min after a meal were increased at week 12, though not to a significant degree. The plasma insulin level peaked 30 min after a meal at both week 0 and 12. AUC_{0-120} of the plasma insulin level at week 12 in group B was higher than that at week 0 (2878.0 ± 1840.5 to 3221.1 ± 2365.3 μU h/mL).

Changes in Plasma Glucagon Level in Meal Tolerance Test at Weeks 0 and 12

There was no significant change in plasma glucagon level or plasma glucagon AUC between week 0 and 12 in either group (Table 2).

Changes in Active GLP-1 and Total GIP Levels in Meal Tolerance Tests at Weeks 0 and 12

Changes in active GLP-1 levels are shown in Figure 4. In group A, the active GLP-1 levels were elevated throughout the experiment at week 12. Among them, active GLP-1 levels 60 and 120 min, and AUC_{0-120} after a meal significantly increased (5.3 ± 0.7 to 7.5 ± 2.7 pmol/L, $P = 0.0039$ and 5.3 ± 0.9 to 6.7 ± 2.7 pmol/L, $P = 0.0332$, and 648.9 ± 91.6 to 843.3 ± 336.9 pmol h/L, $P = 0.0137$, respectively). Active GLP-1 levels peaked 30 min after a meal at week 0 and 60 min after a meal at week 12. In group B, there was no significant difference in

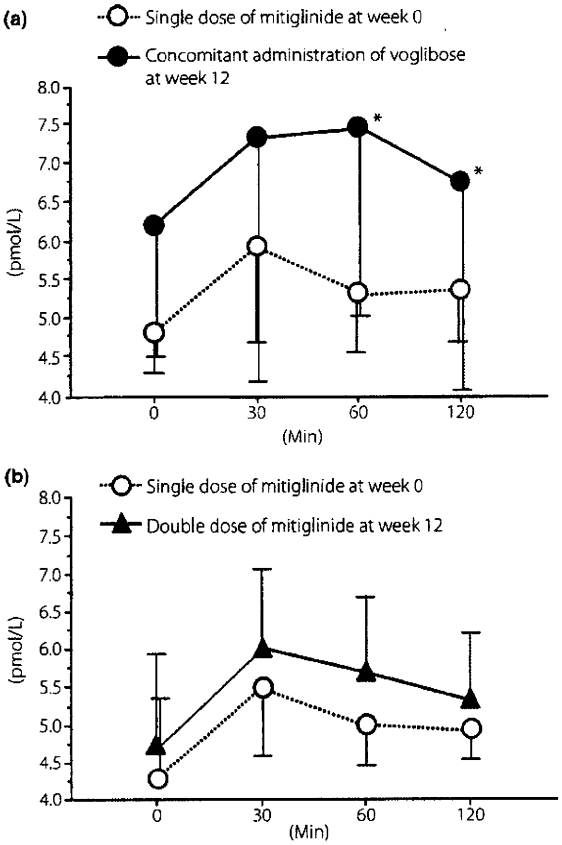

Figure 4 | Change in active glucagon-like peptide-1 level after meal tolerance tests from week 0 to 12 in (a) group A and (b) group B. A significant increase at 60 and 120 min after a meal was observed at week 12 in group A. *$P < 0.05$ *vs* before addition of voglibose (Wilcoxon signed-rank test).

active GLP levels between week 0 and 12. Active GLP-1 levels peaked 30 min after a meal at weeks 0 and 12.

There was no significant change in total GIP levels between week 0 and 12 in either group (Table 2, Figure 4).

DISCUSSION

In the present study, when mitiglinide was given concomitantly with voglibose for 12 weeks, the peak plasma glucose level after a meal decreased significantly and the time required for plasma glucose level to reach the peak value was prolonged (Figure 2a). Although there was no significant difference in AUC_{0-120} of plasma insulin levels, a significant decrease was observed in AUC_{0-30} ($P = 0.0273$), namely during the early phase of insulin secretion (Figure 3). It has been shown that voglibose inhibits the postprandial increase in plasma glucose level and thereby decreases plasma insulin levels.

It was recently reported that the acute postprandial increase in plasma glucose level (postprandial glucose spike) promotes arteriosclerosis and increases the risk of cardiovascular disease, including myocardial infarction[3]. It has also been reported that hyperinsulinemia impairs vascular endothelial function and increases the risk of ischemic heart disease[4]. In the present study, concomitant administration of mitiglinide and voglibose (group A) more markedly inhibited the postprandial glucose spike and decreased insulin secretion than did a single dose of mitiglinide. This suggests that concomitant use of mitiglinide with voglibose might have a beneficial effect in preventing arteriosclerosis.

In group A, the active GLP-1 levels at 60 and 120 min after a meal were significantly increased at week 12 (Figure 4a). This finding is of particular interest, because there has been no previous report of voglibose significantly increasing the active GLP-1 level in patients with type 2 diabetes mellitus.

Active GLP-1 level was reported to increase when voglibose was given to *ob/ob* mice for 3–4 weeks[5]. It appears that continuous administration of voglibose evoked chronic glucose absorption from the small intestine and increased the amount of undigested carbohydrates, which results in constant stimulation of the lower small intestine and the large intestine, thus promoting differentiation and proliferation of GLP-secreting cells (L-cells)[6]. This mechanism of action appears to explain why the GLP-1 levels at 60 and 120 min after a meal were significantly increased at week 12 in group A. These findings suggest that concomitant use of mitiglinide and voglibose could spare excessive insulin secretion, and that the increase in GLP-1 level might protect the function of pancreatic β-cells and regulate postprandial plasma glucose levels.

It has been reported that GLP-1 improved abnormal glucagon secretion, particularly the paradoxical rise in glucagon secretion[7]. However, in the present study, no relationship between GLP-1 secretion and pancreatic glucagon secretion was observed in either group (Table 2). Further investigation is necessary to elucidate whether the beneficial effects of the concomitant use of α-GI and mitiglinide treatment, on better long-term glucose control, would depend on the suppression of glucagon secretion.

In contrast, in group B, HbA_{1c}, GA and 1,5-AG levels significantly improved at week 12 (Table 2). In a double-blind comparative phase III clinical study of mitiglinide in China[8], HbA_{1c} levels improved when the mitiglinide dose was increased from 10 to 20 mg, which is similar to the results of the present study. However, meal tolerance tests at week 12 showed no significant change in plasma glucose level in group B (Figure 2). It is quite difficult to explain the discrepancy; the plasma glucose level 120 min after a meal in group B showed no significant decrease at week 12, but did tend to decrease compared with that of week 0. In the present study, we investigated the plasma glucose levels only until 120 min after a meal. However, there was a great difference in plasma glucose levels at 120 min or later (Figure 2). Therefore, the HbA_{1c} level might have been significantly improved at 120 min or later after a meal in group B.

In the present study, we randomly allocated the subjects to two groups; incidentally, the background characteristics were significantly different between the groups (Table 1). The duration of diabetes was shorter and the blood glucose control was worse in group A participants on entry to the study.

Mean BMI was 26.0 in participants of group A, which shows that they were slightly more obese than the Japanese patients with type 2 diabetes. Because impairment of early insulin secretion is closely related to the pathogenesis of type 2 diabetes in Japanese patients and the secretory capacity of pancreatic β-cells is weaker in Japanese patients than those in the USA and Europe[9–11], concomitant use of mitiglinide with voglibose could be more useful even in fairly well-controlled obese Japanese patients with type 2 diabetes mellitus as long as they were switched to concomitant treatment at an early stage.

In contrast, in group B in the present study, mean BMI was 23.2; that is, they were non-obese, plus their mean duration of diabetes was much longer (12.4 years).

In non-obese Japanese type 2 patients whose blood glucose levels are fairly well controlled with mitiglinide (30 mg/day) alone, a double dose of mitiglinide treatment could be expected to improve the plasma glucose level without causing bodyweight gain and/or hypoglycemia. This might support the potential for a double dose of mitiglinide to still be effective in non-obese, long-standing type 2 diabetes patients with low insulin secretory capacity.

Because the sample size of the present study was limited, a large-scale study should be carried out to confirm the patient benefits by treatment regimen.

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ORIGINAL RESEARCH

Incretin Responses to Oral Glucose Load in Japanese Non-Obese Healthy Subjects

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ABSTRACT

Introduction: Recently, incretin-related therapy has been developed for the new treatment of diabetes mellitus; however, incretin response to glucose ingestion in normal glucose tolerant (NGT) subjects has not been clarified in detail with special reference to the role of incretin hormones, glucagon, and a family history of diabetes. ***Methods:*** We conducted a 75 g oral glucose tolerance test in 30 NGT subjects. ***Results:*** The total glucose-dependent insulinotropic peptide (GIP)-$AUC_{0\text{-}120}$ (area under the curve over a period of 0-120 minutes) was correlated with immunoreactive insulin (IRI)-$AUC_{0\text{-}120}$ ($P<0.05$), insulinogenic index (II; $P<0.05$), ΔIRI between 0 and 120 minutes ($P<0.05$). Active glucagon-like peptide-1 (GLP-1) $AUC_{0\text{-}120}$ was correlated inversely both with Δ glucose between 0 and 30 minutes ($P<0.01$) and with Δ immunoreactive glucagon between 0 and 30 minutes ($P<0.05$). Δ Total GIP between 0 and 15 minutes ($P<0.01$), Δ total GIP between 0 and 30 minutes ($P<0.05$), and the total GIP-$AUC_{0\text{-}120}$ ($P<0.05$) in the subjects with a family history of type 2 diabetes were significantly higher than those in the subjects without a family history. ***Conclusion:*** These results suggest that GIP possibly facilitates insulin secretion in response to oral glucose load directly and active GLP-1 may exert the glucoregulatory action via the suppression of glucagon secretion in NGT subjects. Notably, the subjects with a family history of diabetes exert significantly higher GIP response in the early phase of glucose load compared with those without a family history.

Keywords: diabetes mellitus; glucagon; glucagon-like peptide-1; glucose-dependent insulinotropic polypeptide; incretin; insulin

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INTRODUCTION

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are incretin hormones that are released from enteroendocrine cells within minutes after food intake. GIP is secreted from enteroendocrine cells (K cells) primarily in the proximal small intestine (duodenum and jejunum), while GLP-1 is secreted from enteroendocrine cells (L cells) that are scattered throughout the small bowel (primarily in the distal portion) and colon.[1] Both GIP and GLP-1 have been shown to stimulate insulin secretion in response to glucose and nutrient ingestion.[2] The primary role of GIP is supposed to stimulate the secretion of insulin from pancreatic β cells, especially in the postprandial state. In addition to its insulinotropic effect, GLP-1 has a potent glucagonostatic action, inhibits gastric emptying, decreases food intake, and slows the rate of endogenous glucose production, all of which would ameliorate the blood glucose levels in type 2 diabetes mellitus (T2DM). GLP-1 shows direct effects through GLP-1 receptors in the hypothalamus, thereby regulating the appetite, food intake, and body weight in the "gut-brain axis" (the reciprocal system between the gastrointestinal tract and central nervous system to regulate short- and long-term energy homeostasis).[1]

The earlier studies on the response of both incretin hormones to an oral glucose tolerance test (OGTT) and test meal revealed that the incretin effect of Caucasian patients with T2DM is significantly lower than healthy subjects.[3,4] Conversely, in the three studies demonstrating incretin responses after ingestion of glucose and/or nutrients in Japanese subjects,[5-7] any difference between T2DM patients with or without obesity and nondiabetic control subjects could not be found out.

Whether the abnormality of the incretin response to the nutrient might be involved in the pathophysiological mechanisms of the development of T2DM or not awaits further investigation.

At the first step of the analysis of the mechanism, it is necessary to reveal the response of incretin to glucose or food intake in normal glucose-tolerant (NGT) subjects in detail with special reference to the relationship between incretin hormone responses and insulin, glucagon secretion, gender, metabolic parameters inclusive of body mass index (BMI), waist circumference, and a family history of diabetes in particular.

In this study, the various parameters were investigated after an intake of 75 g glucose in order to clarify the relationship between the responses of incretin hormones and glucose, insulin, or glucagon in 30 healthy subjects who are currently NGT.

MATERIALS AND METHODS

Materials

Thirty healthy subjects, who were a group of medical students within a pregraduate course, participated in this study. All of them had NGT. The exclusion criteria were subjects who had any major illness or premenopausal women who were pregnant or nursing. This study has been approved by the Ethics Committee of Hyogo College of Medicine. All participants provided written informed consent before the start of the study.

Methods

To identify the baseline characteristics of incretin secretion in Japanese subjects, the influence of body weight, BMI, height, gender, and a family

history of diabetes on the hormonal response to oral glucose ingestion were also examined.

The OGTT consisted of ingesting 75 g glucose (Toleran G®, Ajinomoto Pharma, Tokyo, Japan) in the overnight fasting state within 5 minutes. For active GLP-1 and total GIP analysis, catheters were placed in cubital veins and blood samples were withdrawn directly into the blood collection ethylenediaminetetraacetic acid-disodium salt coated tubes (1.25 mg/mL blood) containing aprotinin and an inhibitor of dipeptidyl peptidase-4 (10 μL/mL blood; Linco Research Inc., MO, USA) before the start of the OGTT and 15, 30, 60, and 120 minutes after ingestion, respectively. The levels of plasma glucose, immunoreactive insulin (IRI), immunoreactive glucagon (IRG), total GIP, and active GLP-1 were measured at each time interval. Plasma glucose concentrations were measured immediately by the glucose oxidase method. IRI was measured using chemiluminescent enzyme immunoassay (Fujirebio Inc., Tokyo, Japan). Plasma levels of glucagon were measured using a radioimmunoassay via the glucagon kit daiichi-II (TFB Corp., CA, USA). Plasma levels of active GLP-1 were measured using an enzyme-linked immunosorbent assay (ELISA), using the GLP-1 active ELISA kit (Millipore Corp., MA, USA) according to the manufacturer's instructions. Plasma levels of the total GIP were also measured using an ELISA, using the human GIP (total) ELISA kit (Millipore Corp., MA, USA).

Statistical Analysis

The results of statistical analysis were shown as the mean ± standard deviation (SD) unless otherwise specified. The trapezoidal method was used to calculate all areas under the curves (AUCs). The homeostasis model assessment was used both for assessing insulin resistance (HOMA-R) and insulin secretion (HOMA-β).[8] HOMA-R was calculated as fasting plasma glucose (mg/dL) × fasting serum insulin (μIU/mL)/405. HOMA-β was calculated as (360× fasting serum insulin [μIU/mL]) / (fasting plasma glucose [mg/dL] −63). The insulinogenic index (II) was calculated as the ratio of the increment of serum insulin to plasma glucose concentration 30 minutes after an oral glucose load (IRI $\Delta_{0\text{-}30\ \text{minutes}}$/ glucose $\Delta_{0\text{-}30\ \text{minutes}}$).[9] Single Pearson correlation analysis was used to examine bivariate relationships. The unpaired *t*-test was used when two separate sets of samples (family history [+]/[−]) were obtained. A *P* value of less than 0.05 was taken to indicate statistically significant difference.

RESULTS

As shown in Table 1, the age of subjects was 24.7±2.3 years and BMI was 21.8±3.5 kg/m^2. The average BMI of women (10 subjects) was 19.3 kg/m^2 and men (20 subjects) was 23.1 kg/m^2. The mean plasma glucose, IRI, total GIP, active GLP-1, and IRG levels in the subjects are summarized in Figure 1. The mean plasma glucose and IRI peaked at 30 minutes (Figure 1A).

The mean active GLP-1 peaked at 15 minutes and the peak level of active GLP-1 was 10.6 pmol/L. The level of total GIP increased steeply in 15 minutes, and thereafter increased gradually with time, whereas the IRG gradually decreased during the observation period. The peak level of total GIP was 265.5 pg/mL (Figure 1B).

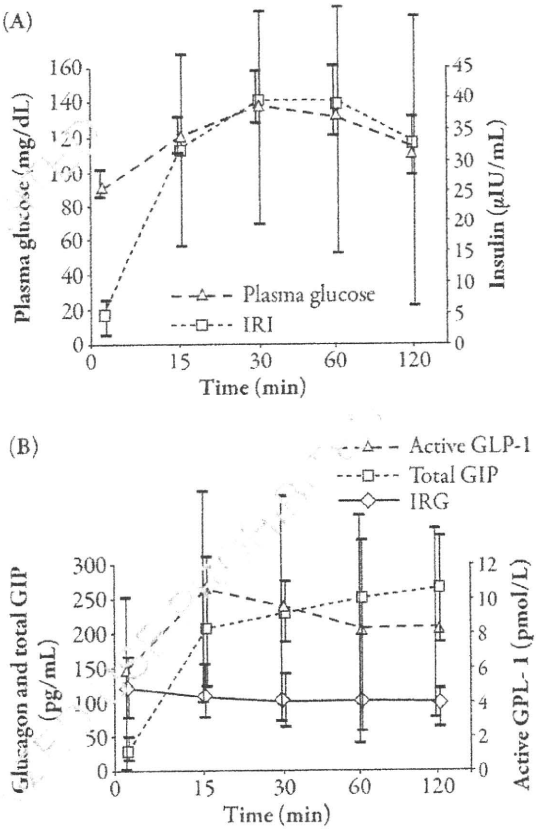
The total GIP-$AUC_{0\text{-}120}$ was related to the IRI-$AUC_{0\text{-}120}$ (R=0.39, P<0.05), II (R=0.40, P<0.05), and Δ IRI between 0 and 120 minutes (R=0.40, P<0.05), as shown in Figure 2A, B, and C, respectively. Conversely, the active GLP-1-$AUC_{0\text{-}120}$ was inversely correlated with Δ glucose between 0 and 30 minutes (R=−0.47,

Table 1. Clinical characteristics of 30 Japanese subjects with normal glucose tolerance.

	Week 0
Age, years	24.7±2.3
Gender, *n*	
Men	20
Women	10
Height, m	1.67±0.08
Body weight, kg	60.8±11.6
BMI, kg/m^2	21.8±3.5
Waist circumference, cm	76.2±11.6
HOMA-R	1.1±0.8
HOMA-β, %	68.3±45.6
Insulinogenic index	0.8±0.5
Family history of diabetes* (presence [+]/absence [−]), *n*	[+]13/[−]17
Of the 13 subjects with the presence of a family history:	
Subjects with first-degree relative, *n*	4
Subjects with second-degree relative, *n*	8
Subjects with third-degree relative, *n*	1

*Defined as subjects with a first- to third-degree relative with T2DM.
BMI=body mass index; HOMA-R=homeostasis model assessment for insulin resistance; HOMA-β=homeostasis model assessment for insulin secretion; T2DM=type 2 diabetes mellitus.

Figure 1. Results of oral glucose tolerance test in (A) plasma glucose and insulin (B) glucagon, total glucose-dependent insulinotropic peptide, and active glucagon-like peptide-1. Data are calculated as mean±SD. GIP=glucose-dependent insulinotropic peptide; GLP-1=glucagon-like peptide-1; IRG=immunoreactive glucagon; IRI=immunoreactive insulin.

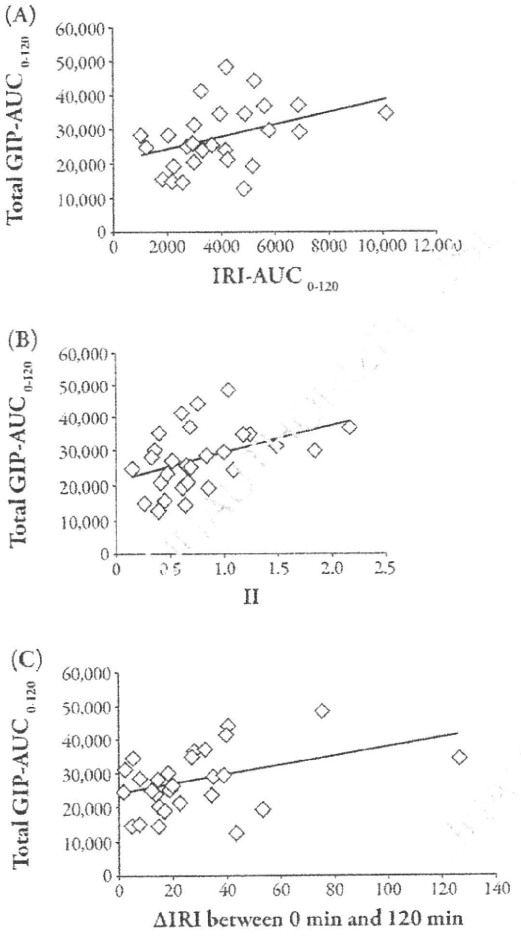


P<0.01) and Δ IRG between 0 and 30 minutes (R=–0.40, P<0.05), as shown in Figure 3A and B, respectively.

Body weight was correlated with HOMA-R (R=0.50, P<0.01), II (R=0.40, P<0.05), and HOMA-β (R=0.37, P<0.05). BMI was correlated with HOMA-R (R=0.50, P<0.01) and HOMA-β (R=0.37, P<0.05), and waist circumference was correlated with HOMA-R (R=0.44, P<0.05) and HOMA-β (R=0.39, P<0.05). However, body weight, BMI, waist circumference, height, and gender were not related to the secretory response of total GIP (Δ GIP between 0 minutes and 15, 30, 60, 120 minutes, and GIP-AUC$_{0\text{-}120}$), active GLP-1 (Δ active GLP-1 between 0 minutes and 15, 30, 60, 120 minutes, and active GLP-1-AUC$_{0\text{-}120}$), and IRG (Δ IRG between 0 minutes and 15, 30, 60, 120 minutes, and IRG-AUC$_{0\text{-}120}$).

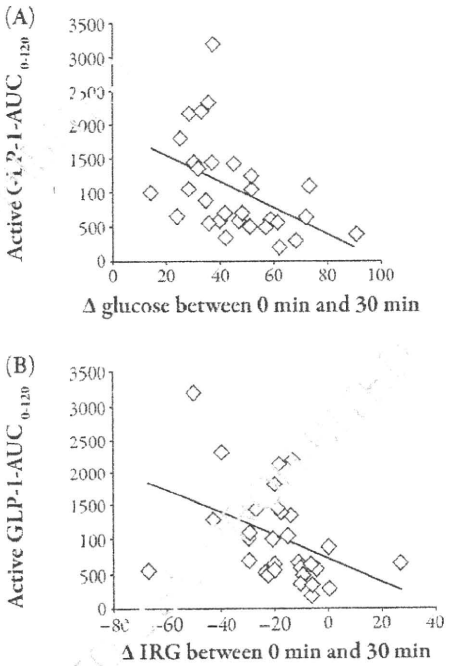
The presence or absence of T2DM family history was not correlated with BMI, waist circumference, HOMA-R, II, HOMA-β, and IRI-AUC$_{0\text{-}120}$ as shown in Table 2. It was not correlated with the secretory response of active GLP-1 (Δ active GLP-1 between 0 minutes and 15, 30, 60, 120 minutes, and active GLP-1-AUC$_{0\text{-}120}$) and IRG (Δ IRG between 0 minutes and 15, 30, 60, 120 minutes, and IRG-

Figure 2. (A) Correlation between total glucose-dependent insulinotropic polypeptide (GIP) area under the curve $(AUC)_{0\text{-}120}$ and immunoreactive insulin (IRI)-$AUC_{0\text{-}120}$ (y=1.797x+205.79; R=0.39; P<0.05). (B) Correlation between total GIP-$AUC_{0\text{-}120}$ and insulinogenic index (y=7086.1x+21,774; R=0.40; P<0.05). (C) Correlation between total GIP-$AUC_{0\text{-}120}$ and ΔIRI between 0 and 120 minutes (y=140.35x+24,012; R=0.40; P<0.05). AUC=area under the curve; GIP=glucose-dependent insulinotropic peptide; II=insulinogenic index; IRI=immunoreactive insulin.



$AUC_{0\text{-}120}$), respectively. However, Δ total GIP between 0 and 15 minutes (R=0.54, P<0.01), Δ total GIP between 0 and 30 minutes (R=0.39, P<0.05), and the total GIP-$AUC_{0\text{-}120}$ (R=0.39, P<0.05) in subjects with a family history of diabetes were significantly higher

Figure 3. (A) Correlation between active glucagon-like peptide-1 (GLP-1) area under the curve $(AUC)_{0\text{-}120}$ and Δglucose between 0 and 30 minutes (y=−19.551x +1920.9; R=−0.47; P<0.01). (B) Correlation between active GLP-1-$AUC_{0\text{-}120}$ and Δ immunoreactive glucagon (IRG) between 0 and 30 minutes (y=−16.545x+720.37; R=−0.40; P<0.05). AUC=area under the curve; GIP=glucose-dependent insulinotropic peptide; IRG=immunoreactive glucagon.



than those subjects without a family history of diabetes. In order to confirm this, unpaired t-tests were used to examine whether there was a difference between the Δ total GIP between 0 and 15 minutes, Δ total GIP between 0 and 30 minutes, and GIP-$AUC_{0\text{-}120}$ in subjects with a family history of diabetes or no family history of diabetes. Total GIP-$AUC_{0\text{-}120}$ (P=0.039) in subjects with a family history of diabetes were significantly higher than those subjects without a family history of diabetes, and as shown in Figure 4, Δ total GIP between 0 and 15 minutes (P=0.0032) and Δ total GIP between 0 and 30 minutes (P=0.041) in subjects with a family history of

Table 2. Clinical characteristics of the enrolled subjects with or without a family history of diabetes.

	Family history of diabetes*		*P* value
	Presence (+)	Absence (–)	
Number of subjects	13	17	
Age, years	24.8±2.7	24.5±1.9	NS
BMI, kg/m^2	21.2±3.4	22.3±3.6	NS
Waist circumference, cm	73.8±12.6	78.1±10.8	NS
HOMA-R	1.1±0.9	1.2±0.6	NS
HOMA-β, %	64.3±49.8	71.4±43.4	NS
Insulinogenic index	0.8±0.5	0.8±0.5	NS
IRI-AUC$_{0\text{-}120}$, μU h/mL	3840.2±1493.0	4398.4±2321.5	NS

*Defined as subjects with first- to third-degree relatives with T2DM.
AUC=area under the curve; BMI=body mass index; HOMA-R=homeostasis model assessment for insulin resistance; HOMA-β=homeostasis model assessment for insulin secretion; IRI=immunoreactive insulin; NS=nonsignificant; T2DM=type 2 diabetes mellitus.

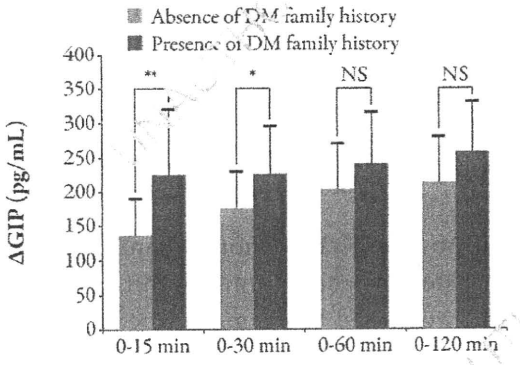


Figure 4. Correlation of Δglucose-dependent insulinotropic polypeptide (GIP) and a family history of type 2 diabetes mellitus (T2DM), defined as having a first- to third-degree relative with T2DM. **P<0.01. *P<0.05. DM=diabetes mellitus; GIP=glucose-dependent insulinotropic peptide; NS=nonsignificant.

diabetes were significantly higher than those subjects without a family history of diabetes.

DISCUSSION

The analytical data of incretin responses and kinetics of healthy Japanese subjects are scant;[5-7] therefore, in the present study 30 Japanese NGT subjects were recruited.

Nauck et al.[10] administered synthetic human GIP, GLP-1, and placebo under hyperglycemic clamp conditions in nine T2DM and nine age- and weight-matched NGT subjects. Both GIP and GLP-1 (7-36 amide) dose dependently augmented insulin secretion in both the T2DM and NGT group.[10] Cataland et al.[11] gave 75 g glucose to 21 healthy volunteers, and their serum GIP levels increased after oral glucose ingestion.

The present study shows that the total GIP-AUC$_{0\text{-}120}$ in healthy Japanese subjects positively correlated with the IRI-AUC$_{0\text{-}120}$ (P<0.05), II (P<0.05), and Δ IRI between 0 and 120 minutes (P<0.05). The present study has demonstrated that GIP clearly augmented insulin secretion in response to an oral glucose load in healthy subjects, which suggests that the secretory response of total GIP, which is secreted from K cells primarily in the proximal intestine after glucose ingestion, could be a potent trigger and/or stimulant of insulin secretion in healthy subjects.

Conversely, active GLP-1-AUC$_{0\text{-}120}$ was not related to IRI-AUC$_{0\text{-}120}$ and II. Active GLP-1 did not show any significant augmentation of insulin secretion in Japanese NGT subjects.

However, active GLP-1-$AUC_{0\text{-}120}$ was correlated inversely both with Δ glucose between 0 and 30 minutes ($P<0.01$) and with Δ IRG between 0 and 30 minutes ($P<0.05$). This means that GLP-1 may exert its glucoregulatory action via the suppression of glucagon secretion rather than the stimulation of insulin secretion in healthy Japanese subjects. This effect of GLP-1 possibly contributes to lower the plasma glucose level as a result of reduction in hepatic glucose output.[1] The secretory response of GLP-1 has been reported to be decreased in T2DM,[3,4,12] which could weaken the incretin effect of GLP-1 on the reduction in hepatic glucose output.

In addition, GLP-1 may also lower plasma glucose levels by retarding gastric emptying, because there is an inverse relationship between gastric emptying of glucose and plasma GLP-1.[13] To establish the precise mechanism of the inverse relationship between GLP-1 secretion and plasma glucose, the function of gastric emptying of glucose needs to be examined.

The key factors regulating the GIP/GLP-1 response after glucose ingestion by NGT and abnormal glucose tolerant subjects are not well understood. Vollmer et al.[14] reported that female gender was positively related to GLP-1 concentration. Carroll conducted a study to determine the influence of BMI and gender on postprandial hormone responses such as insulin, leptin, ghrelin, active GLP-1, and glucagon. He found that men had significantly greater fasting ($P=0.02$) and postprandial ($P=0.03$) glucagon, and men tended to have higher GLP-1 concentrations ($P=0.06$). Obese subjects had higher fasting glucose and insulin concentrations, while BMI did not affect the postprandial GLP-1 response.[15] Several studies have demonstrated that gender did not influence glucagon responses to stimuli such as exercise and hypoglycemia.[16-18] Therefore, in the present study, the influence of gender, height, body weight, BMI or waist circumference on the incretin hormones and glucagon in healthy Japanese subjects was examined. However, no significant relationship among these factors in Japanese NGT subjects was found. Neither gender nor physical constitution affected the secretion of incretin hormones in the Japanese subjects.

In addition, we examined whether a family history of diabetes (presence or absence of T2DM patients within the third degree of kinship) was correlated with the level and/or the response of incretin hormones, because Japanese patients with T2DM usually have impaired insulin secretion even in the early phase of diabetes rather than insulin resistance.[19] In earlier studies in Western countries, the incretin effect in relatives of T2DM patients has been examined, and it is still controversial. In a German study, a lower insulin secretory response to exogenous GIP in first-degree relatives of patients with T2DM was shown,[20] and in a Danish study, the daytime (after meals) AUC for plasma GIP was significantly increased in the relatives of diabetes patients compared with control subjects without any family history of diabetes.[21] However, in another German study, the incretin effects were similar in first-degree relatives of patients with T2DM and healthy control subjects.[22] As there had been no attempt to reveal the relationship between a family history of diabetes and incretin response in Japanese subjects, we enrolled Japanese subjects who were NGT with or without a family history of diabetes. Interestingly, we found that Δ total GIP between 0 and 15 minutes ($R=0.54$, $P<0.01$), Δ total GIP between 0 and 30 minutes ($R=0.39$, $P<0.05$), and the total GIP-$AUC_{0\text{-}120}$ ($R=0.39$, $P<0.05$) in the subjects with a family history of diabetes were significantly higher than those in the subjects without a family history of diabetes. Conversely, IRI, HOMA-R, HOMA-β, II, active GLP-1, and IRG were not correlated

with the presence or absence of a family history of diabetes.

It has been speculated that the loss of insulinotropic action of GIP in T2DM might occur as a result of either chronic desensitization of GIP receptors,[23] or a reduction in the expression of GIP receptors on pancreatic β cells.[24,25] Both abnormalities of GIP receptors might have already been induced in an NGT state with the presence of a family history, and a hypersecretion of GIP could potentially compensate for a reduced insulinotropic effectiveness of GIP, which could enhance insulin secretion particularly in the early phase in response to a glucose load.

Another hypothesis to account for the hypersecretion of GIP in NGT subjects with T2DM family history is the rapid gastric emptying. Since Phillips et al.[26] reported that recently diagnosed T2DM patients emptied their stomach more rapidly than nondiabetic control subjects, in the subjects with a family history of T2DM, the GIP secretion from the upper small intestine may already be augmented by rapid gastric emptying even in an NGT state. Furthermore, whether the background mechanism of the phenomenon would depend on the differences in the rate of glucose absorption in the small intestine and density of K cells should be examined in the future.

The augmented response of GIP after glucose load in subjects with a family history of T2DM in the present study could be related, in part, to the onset mechanism of Japanese T2DM patients, although further study is required. The low number of subjects is acknowledged as a limitation of the study.

In conclusion, the present study was designed to investigate the secretory patterns of two incretin hormones to OGTT and the relationship between incretin hormones and glucose, insulin, or glucagon response in healthy Japanese subjects. The results demonstrate that, at least in subjects enrolled in the study, GIP from the upper small intestine may play a role as an insulinotropic hormone and GLP-1 may play a role as a glucagonostatic hormone collaboratively. Furthermore, healthy subjects with a family history of diabetes who have normal glucose tolerance exert a significantly higher GIP response, especially in the early phase of GIP secretion in response to an oral glucose load compared with those without a family history of T2DM.

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The authors declare that there is no duality of interest associated with this manuscript.
Dr. Namba is the guarantor for this article, and takes responsibility for the integrity of the article as a whole.

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Effects of gliclazide on platelet aggregation and the plasminogen activator inhibitor type 1 level in patients with type 2 diabetes mellitus

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Abstract

Vascular complications are a common factor determining morbidity and mortality of diabetic patients. In vitro studies have revealed that gliclazide has antiplatelet activities. To clinically assess this action, we measured the effects of gliclazide on platelet activities and abnormal fibrinolysis in patients with type 2 diabetes mellitus. We studied 14 patients aged 38 to 72 years (9 men and 5 women) with type 2 diabetes mellitus who have been treated with glibenclamide in our hospital for more than 6 months. We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg. We titrated the dose of gliclazide to keep the glycemic control at the same level as the previous (glibenclamide) treatment. We measured 10 μmol/L serotonin–induced or 0.5 μmol/L adenosine diphosphate (ADP)–induced platelet aggregate formation by particle counting using light scattering at baseline and up to 6 months after the switch. After switching to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced ($P < .05$, compared with the levels observed after glibenclamide treatment). The body mass index, fasting plasma glucose, immunoreactive insulin, homeostasis model assessment of insulin resistance, hemoglobin A_{1c} (HbA_{1c}), total cholesterol, triglycerides, high-density lipoprotein cholesterol, prothrombin time, activated partial thromboplastin time, fibrinogen, thrombin-antithrombin III complex, plasmin-α2-plasmin inhibitor complex, and plasma plasminogen activator inhibitor type 1 (PAI-1) were not changed. In the group with improved HbA_{1c} (n = 5), ADP-induced platelet aggregate formation and plasma PAI-1 level were significantly reduced ($P < .05$, compared with the group with aggravated HbA_{1c}, n = 9). Multiple regression analysis showed that percentage change of ADP-induced platelet aggregate formation (standardized $\beta = 0.540$, $P < .05$) was independently associated with percentage change of plasma PAI-1 level in addition to percentage change of HbA_{1c} (standardized $\beta = 0.657$, $P < .05$) ($R = 0.939$, $P < .05$) after switching to gliclazide. The other independent variants, like the final dose of gliclazide, homeostasis model assessment of insulin resistance, percentage change of prothrombin time, activated partial thromboplastin time, and total cholesterol, were not significantly associated with the percentage change of plasma PAI-1 level. These results indicate that gliclazide inhibits platelet aggregation via the serotonin pathway, independently of the metabolic control per se. Furthermore, in the patients with improved glycemic control, gliclazide could inhibit ADP-induced platelet aggregation and reduce PAI-1 level. Taken together, the results show that gliclazide may be more useful for the prevention of diabetic vascular complications than glibenclamide.

1. Introduction

Atherosclerotic complications play a crucial role in the prognosis of type 2 diabetes mellitus (DM). It is fully recognized that long-term macrovascular complications are common factors determining morbidity and mortality in the diabetic population. The Diabetes Control and Complications Trial and UK Prospective Diabetes Study indicate a consistent relationship between hyperglycemia and the incidence of chronic vascular complications in type 1 and type 2 DM, respectively [1,2]. Platelet function in DM patients is enhanced and is correlated with both agonist-induced and spontaneous aggregation [3]. It is thought that long-term exposure to high glucose levels may enhance

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Table 1
Characteristics of enrolled patients

Age (y)	61.5 ± 2.6
Sex (M/F)	9/5
Height (cm)	165.3 ± 2.9
Weight (kg)	62.0 ± 3.1
BMI (kg/m^2)	22.5 ± 0.8
Duration of DM (y)	12.0 ± 1.8
HbA_{1c} (%)	7.4 ± 0.2
FPG (mg/dL)	165.0 ± 7.0
IRI (μU/mL)	7.8 ± 1.6
HOMA-R	3.0 ± 0.6

Data are expressed as mean ± SEM.

platelet function in DM patients. Moreover, rapid alterations of platelet aggregability in acute hyperglycemia have also been reported [4]. Intraplatelet serotonin (5-hydroxytryptamine; 5-HT) content is diminished and plasma levels of 5-HT are increased in DM patients [5].

This increase in plasma 5-HT may reflect enhanced release of platelet 5-HT by hyperactive platelets that may contribute to the pathogenesis of atherosclerosis. The measurement of 5-HT–induced platelet aggregation is therefore a useful method to evaluate the risk of diabetic complications in DM patients [5].

A technique for studying platelet aggregation by particle counting using light scattering may detect subtle changes in platelet activation [6]. Hypercoagulability and decreased fibrinolysis, including increased plasma plasminogen activator inhibitor type 1 (PAI-1) level, are often found and are considered to be risk factors of cardiovascular diseases and glucose intolerance, especially in patients with non–insulin-dependent DM [7]. Gliclazide is a second-generation sulfonylurea with the potency of free radical scavenger activity. Some studies have shown that gliclazide has beneficial effects on the hemorrheologic abnormalities seen in diabetic vascular disease [8-12].

To assess this clinically, we measured platelet activities and fibrinolysis in patients with type 2 DM treated with gliclazide; and we compared the results with those obtained in patients treated with glibenclamide.

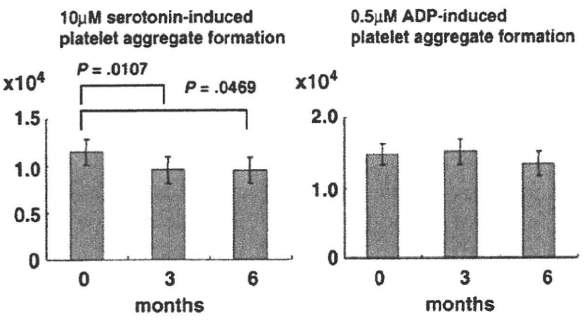

Fig. 1. Effects of gliclazide on platelet aggregation. Data are expressed as mean ± SEM.

2. Subjects and methods

2.1. Subjects

Fourteen patients with type 2 DM (9 men and 5 women; age [mean ± SEM], 61.5 ± 2.6) were randomly chosen as subjects. They were admitted to our metabolic ward between the years 2001 and 2002. Diagnosis of diabetes was based on World Health Organization 1998 criteria. All patients were treated with diet and glibenclamide. All the procedures in the study and the protection of the patients' private information were approved by the ethical committee of Hyogo College of Medicine. Informed consent was obtained from each patient before enrollment in the study.

2.2. Experimental protocol

We switched from glibenclamide to gliclazide using the average ratio of the respective doses, 2.5 vs 40 mg (1.25-20 mg in 2 patients, 2.5-40 mg in 6 patients, 5.0-80 mg in 2 patients, and 7.5-120 mg in 3 patients). We titrated the dose of gliclazide to keep the glycemic control at the same level as in the glibenclamide control. Patients' blood was assayed 3 times: before switching from glibenclamide to gliclazide and then 3 and 6 months after switching.

2.3. Blood sample preparation

Blood was collected in fasting condition on the respective mornings. Venous blood was drawn into 3.8% sodium citrate (1:9 vol/vol). Platelet-rich plasma (PRP) and platelet-poor plasma were obtained by centrifugation of the citrated blood at room temperature for 10 minutes at 150g and for 15 minutes at 3000g, respectively. The platelet count in PRP was adjusted to 2×10^{11}/L with platelet-poor plasma.

For the measurement of PAI-1, blood was centrifuged for 15 minutes at 3000g; and the supernatant was kept at −80 °C until assayed. Fasting plasma glucose (FPG), immunoreactive insulin (IRI), hemoglobin A_{1c} (HbA_{1c}), fasting serum concentrations of total cholesterol (T-Chol), triglycerides (TG), high-density lipoprotein cholesterol (HDL-Chol), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen (Fbg), thrombin-antithrombin III complex (TAT), and plasmin-α2-plasmin inhibitor complex (PIC) were also measured. Total cholesterol, TG, and HDL-

Table 2
Effects of gliclazide on metabolic factors

	Before	3 mo	6 mo
BMI (kg/m^2)	22.5 ± 0.8	22.3 ± 0.8	21.9 ± 0.8
HbA_{1c} (%)	7.4 ± 0.2	8.0 ± 0.3	7.7 ± 0.3
FPG (mg/dL)	165.0 ± 7.0	166.0 ± 9.9	170.0 ± 8.0
IRI (μU/mL)	7.8 ± 1.6	7.5 ± 1.5	7.4 ± 1.1
HOMA-R	3.0 ± 0.6	3.0 ± 0.8	3.0 ± 0.4
T-Chol (mg/dL)	205.0 ± 8.3	205.0 ± 8.7	201.0 ± 9.4
TG (mg/dL)	121.0 ± 22.1	102.0 ± 10.6	135.0 ± 19.3
HDL-Chol (mg/dL)	50.0 ± 2.6	50.0 ± 2.3	48.0 ± 1.8

Table 3
Effects of gliclazide on coagulation test and PAI-1

	Before	3 mo	6 mo
PT-INR	0.93 ± 0.01	0.92 ± 0.01	0.92 ± 0.02
APTT (s)	25.8 ± 0.7	25.8 ± 0.6	26.6 ± 0.4
Fbg (mg/dL)	300.0 ± 20.7	301.0 ± 9.5	340.0 ± 11.8
TAT (ng/mL)	50.2 ± 22.2	15.3 ± 5.4	25.8 ± 7.8
PIC (μg/mL)	0.8 ± 0.1	0.8 ± 0.1	1.3 ± 0.3
PAI-1 (ng/mL)	42.0 ± 5.6	35.4 ± 4.9	36.4 ± 5.3

Data are expressed as mean ± SEM. INR indicates international normalized ratio.

Chol were assayed using an autoanalyzer (JCA-BM 2250; Nihon Denshi, Akishima, Tokyo, Japan), while HbA_{1c} was measured by high-performance liquid chromatography (HLC-723G7 system; Tosoh, Tokyo, Japan). The subjects were then divided into 2 groups depending on whether their HbA_{1c} levels were improved or aggravated 6 months after switching from glibenclamide to gliclazide.

2.4. Platelet aggregation

Platelet aggregation was monitored with an AG10 aggregometer (Kowa, Tokyo, Japan) that determines the size and number of platelet aggregates based on particle counting using light scattering [6,13]. A laser beam (675 nm) is passed through a platelet suspension, and the intensity of light scattering provides information on the number and size of aggregates. Data were recorded as a 2-dimensional graph showing the change over time of total light intensity expressed as cumulative summation. The total light intensities of small aggregates were determined. Particles with an intensity of 25 to 400 mV represent small aggregates consisting of less than 100 platelets. Platelet-rich plasma (180 μL) was placed in a cuvette and incubated for 3 minutes at 37°C while rotating at 1000 rpm. Subsequently, 20 μL of 5-HT (final concentration, 10 μmol/L) or adenosine diphosphate (ADP) (0.5 μmol/L) was added; and the formation of platelet aggregates was monitored for 5 minutes. For this experiment, we determined the peak level of aggregate formation.

2.5. Statistical analysis

Values are presented as means ± SEM. Correlations were assessed using Spearman rank correlation test. Multiple regression analysis was performed to assess the combined influence of variables on percentage change of plasma PAI-1 levels. The Wilcoxon signed rank test or the Mann-Whitney U test were used for comparison. Differences were considered significant at $P < .05$. All the statistical analyses were performed using StatView J-5.0 software (SAS Institute, Berkeley, CA).

3. Results

3.1. Clinical characteristics of the patients

The clinical characteristics of the enrolled patients in the study are summarized in Table 1. The mean FPG was 165.0 ± 7.0 mg/dL (reference range, 70-110 mg/dL), and HbA_{1c} was 7.4% ± 0.2% (reference range, 4.0%-5.4%).

3.2. Change of platelet aggregation and metabolic factors after switching to gliclazide

After switching from glibenclamide to gliclazide, platelet aggregate formation induced by serotonin was significantly reduced (P = .0107, compared with glibenclamide treatment) after 3 months and (P = .0469, compared with glibenclamide treatment) after 6 months, although the ADP-induced platelet aggregate formation was not changed at all (Fig. 1). The switch from glibenclamide to gliclazide did not modify body mass index (BMI), FPG, IRI, homeostasis model assessment of insulin resistance (HOMA-R), HbA_{1c}, T-Chol, TG, and HDL-Chol (Table 2).

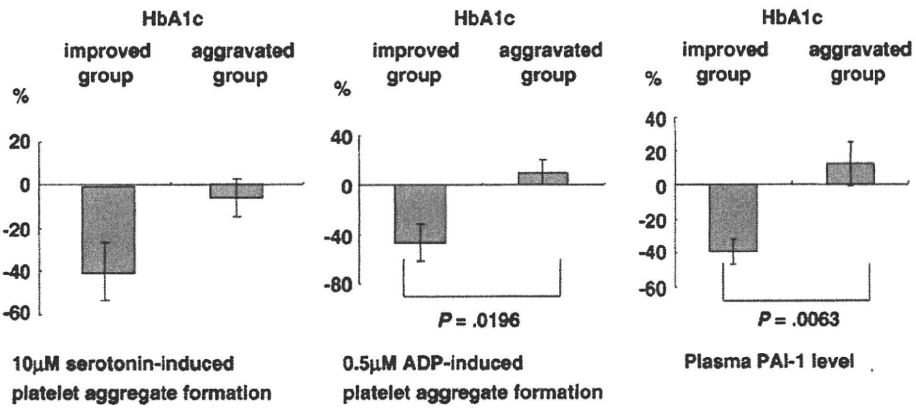

Fig. 2. Percentage change of platelet aggregate formations by 10 μmol/L serotonin or 0.5 μmol/L ADP and plasma PAI-1 level depend on the change of glycemic control after switching to gliclazide. Data are expressed as mean ± SEM.