SHORT REPORT

Synergistic effect of α-glucosidase inhibitors and dipeptidyl peptidase 4 inhibitor treatment

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ABSTRACT

Monotherapy of α-glucosidase inhibitor (α-GI) or dipeptidyl peptidase 4 (DPP4) inhibitor does not sufficiently minimize glucose fluctuations in the diabetic state. In the present study, we evaluated the combined effects of various of α-GI inhibitors (acarbose, voglibose or miglitol) and sitagliptin, a DPP4 inhibitor, on blood glucose fluctuation, insulin and active glucagon-like peptide-1 (GLP-1) levels after nutriment loading in mice. Miglitol and sitagliptin elicited a 47% reduction ($P < 0.05$) of the area under the curve of blood glucose levels for up to 2 h after maltose-loading, a 60% reduction ($P < 0.05$) in the range of blood glucose fluctuation, and a 32% decrease in plasma insulin compared with the control group. All three of the combinations elicited a 2.5–4.9-fold synergistic increase in active GLP-1 ($P < 0.05$ *vs* control). Thus, combined treatment with the α-GI miglitol, which more strongly inhibits the early phase of postprandial hyperglycemia, and DPP4 inhibitor yields both complementary and synergistic effects, and might represent a superior anti-hyperglycemic therapy. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2010.00081.x, 2011)

KEY WORDS: Postprandial hyperglycemia, Insulin, Glucagon-like peptide-1

INTRODUCTION

Although active intervention for postprandial hyperglycemia by acarbose, an α-glucosidase inhibitor (α-GI), prevents development of cardiovascular events[1], it is also important to flatten the postprandial glucose fluctuation to prevent macroangiopathy. α-GI not only inhibits the rapid elevation of postprandial blood glucose level without excessive insulin secretion, but also enhances active glucagon-like peptide-1 (GLP-1) secretion[2]. In contrast, the dipeptidyl peptidase 4 (DPP4) inhibitor, sitagliptin, increases insulin secretion and reduces late-phase elevation of postprandial blood glucose level. We hypothesized that a combination of α-GI and sitagliptin might yield a greater minimizing effect on blood glucose fluctuation while conserving insulin secretion and enhancing active GLP-1 secretion to prevent atherosclerosis[3–5]. Recently, the combined effects of voglibose, an α-GI, and a DPP4 inhibitor on plasma insulin and active GLP-1 levels were reported in mice[6,7]. However, comparison of the combined effect of various α-GIs and sitagliptin on blood glucose fluctuation has not been reported.

In the present study, we showed that combination therapy of α-GIs and sitagliptin can yield complementary and synergistic beneficial effects in mice.

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MATERIALS AND METHODS

Because α-GIs are inhibitors of α-glucosidase, which converts disaccharide to glucose, 7–9-week-old male C57BL/6J mice (Charles River Japan, Tokyo, Japan) were subjected to an overnight fast and orally loaded with 2.5 g/kg of maltose. Blood was collected from the end of the tail just before loading until 2 h after loading, and blood glucose level was measured using the glucose dehydrogenase method. The area under the curve of blood glucose levels for up to 2 h after maltose-loading ($\Delta AUC_{0-2\ h}$) was calculated using the trapezoid method. The range of blood glucose fluctuation was determined as the difference between the maximal and minimal blood glucose levels for up to 2 h.

To evaluate initial insulin-secreting capacity, plasma insulin concentration in blood collected from the end of the tail at 0.25 h after loading was measured using an ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan).

Ten-week-old male C57BL/6J mice freely fed a high-fat diet (D12492 Rodent Diet; Research Diets, New Brunswick, NJ, USA) for 6 weeks were orally loaded with 10 mL/kg of enteral nutrition (Ensure H; Abbot Japan, Tokyo, Japan) to stimulate GLP-1 secretion. To analyze the delayed effect of GLP-1 secretion by α-GI, blood was collected after 0.5 h from the abdominal vein using a syringe containing diprotin A and EDTA at a final concentration of 3 mmol/L and 0.15%, respectively. The concentration of plasma active GLP-1 was measured using an ELISA kit (Millipore Corporation, Billerica, MA, USA).

α-Glucosidase inhibitors (acarbose 10 mg/kg, voglibose 0.1 mg/kg or miglitol 3 mg/kg) was given orally at the time of maltose or enteral nutrition-loading, and sitagliptin (0.3 mg/kg)

was given 0.5 h before oral loading. Test doses of α-GIs were determined from the ED_{50} doses of the inhibition effect on sucrose or maltose-loading in normal rats, respectively.

The experiment was carried out according to the guidelines of Gifu University. Results are expressed as mean ± SD. Significance of difference among groups was analyzed using Dunnett's or Tukey's multiple comparison test based on ANOVA. *P*-values of <0.05 were considered to show statistical significance.

RESULTS

In the maltose-loading test, treatment with each of the three α-GIs alone significantly suppressed the blood glucose peak level at 0.5 h. Administration of miglitol alone significantly suppressed the blood glucose elevation as early as 0.25 and 0.5 h, and delayed the blood glucose peak until 1 h after loading. With administration of sitagliptin alone, the blood glucose level peaked at 0.25 h and was significantly lower at 0.5 and 1 h. Consequently, a complementary suppression of blood glucose elevation was observed at 0.25, 0.5 and 1 h in the combined miglitol and sitagliptin group (Figure 1c). As a result, the $\Delta AUC_{0-2\,h}$ of the blood glucose concentration of the group receiving miglitol alone was almost equal to that with acarbose or voglibose alone. The rate of decrease of the $\Delta AUC_{0-2\,h}$ of the blood glucose concentration in the miglitol and sitagliptin combination group was 47% ($P < 0.05$) compared with the control group, which was larger than that observed in the combined groups with acarbose or voglibose at the tested doses (Table 1). Similarly, combined treatment of miglitol and sitagliptin showed a 60% reduction ($P < 0.05$) of the range of blood glucose fluctuation compared with the control group, and also a significant decrease compared with miglitol or sitagliptin alone (Table 1).

Enhancement of plasma insulin occurred with sitagliptin at 0.25 h after loading (1.6–2.0-fold). In contrast, miglitol alone or combined treatment with miglitol and sitagliptin decreased the plasma insulin concentration by 39 or 32% compared with the control group, respectively (Table 1).

When enteral nutrition was orally loaded in mice fed a high-fat diet, the plasma active GLP-1 concentration was increased by 1.5–1.9-fold after administration of sitagliptin compared with the control group. In contrast, a synergistic increase in the GLP-1 concentration was observed after treatment with a combination of α-GI and sitagliptin (2.5–4.9-fold, $P < 0.05$ *vs* control; Table 1).

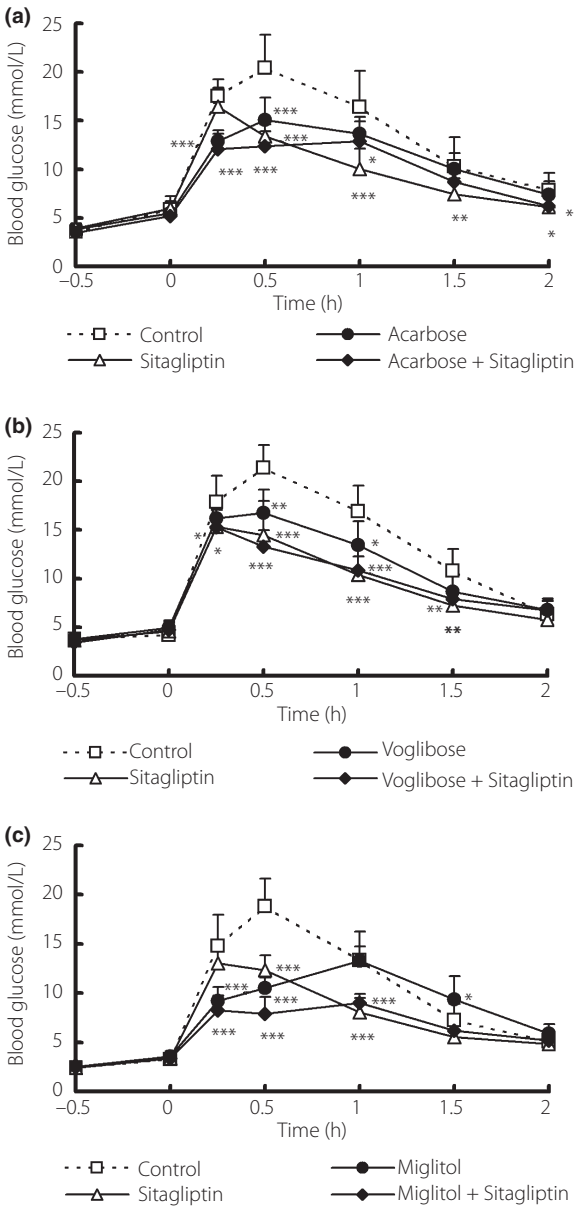

Figure 1 | Blood glucose profiles after oral administration of α-glucosidase inhibitor (α-GI) and sitagliptin (0.3 mg/kg) in maltose-loaded normal mice. (a) Acarbose, 10 mg/kg; (b) voglibose, 0.1 mg/kg; (c) miglitol, 3 mg/kg. Each value represents mean ± SD of 8–10 mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs* the control group using to Dunnett's multiple comparison test.

DISCUSSION

Recently, suppression of postprandial hyperglycemia has come to be considered important for prevention of atherosclerosis[3]. Repeated episodes of blood glucose fluctuation accelerate the adhesion of monocytes to vascular endothelial cells and enhance the development/progression of atherosclerosis[8]. In the present study, the combined administration of α-GI and sitagliptin complementarily lowered the blood glucose level. Furthermore, the combination of miglitol with sitagliptin significantly minimized blood glucose fluctuations. Thus, such combined treatment should reduce the risk of atherosclerosis.

The suppression of postprandial hyperglycemia by α-GI alone and combination treatment with sitagliptin enables insulin secretion to be conserved, and therefore can be expected to mitigate dysfunction of pancreatic β-cells[9]. In contrast, sitagliptin decreases the blood glucose level by enhancing insulin secretion. Thus, long-term administration of this agent might create a

Table 1 | Incremental blood glucose, range of glucose fluctuation and plasma insulin in maltose-loaded mice and plasma active glucagon-like peptide-1 in enteral nutrition-loaded mice

Test compound	Blood glucose concentration $\Delta AUC_{0-2\ h}$		Range of glucose fluctuation	Plasma insulin at 15 min after maltose loading	Plasma active GLP-1 at 30 min after EN loading
	(h*mmol/L)	Inhibition rate (%)	mmol/L	pmol/L	pmol/L
Control	20.9 ± 4.6^{a}	–	15.3 ± 2.6^{a}	111.9 ± 44.8^{ab}	2.18 ± 0.90^{a}
Acarbose, 10 mg/kg	15.6 ± 2.6^{b}	25.4	10.0 ± 2.5^{b}	89.5 ± 25.8^{a}	2.37 ± 0.76^{a}
Sitagliptin, 0.3 mg/kg	12.3 ± 3.3^{b}	41.1	10.9 ± 1.7^{b}	173.9 ± 53.4^{b}	3.33 ± 1.44^{a}
Combination	13.8 ± 2.3^{b}	34.0	8.5 ± 2.3^{b}	118.8 ± 68.9^{ab}	10.76 ± 6.34^{b}
Control	20.9 ± 3.2^{a}	–	17.2 ± 2.4^{a}	113.7 ± 48.2^{a}	2.23 ± 1.86^{a}
Voglibose, 0.1 mg/kg	16.1 ± 3.2^{b}	23.0	12.3 ± 1.7^{b}	111.9 ± 17.2^{a}	2.78 ± 1.23^{a}
Sitagliptin, 0.3 mg/kg	12.7 ± 2.9^{b}	39.2	11.6 ± 2.4^{b}	229.0 ± 93.0^{b}	3.54 ± 2.33^{ac}
Combination	13.6 ± 1.3^{b}	34.9	10.7 ± 1.8^{b}	142.9 ± 43.1^{a}	5.52 ± 2.34^{bc}
Control	17.9 ± 3.6^{a}	–	15.5 ± 2.6^{a}	158.9 ± 44.8^{a}	2.37 ± 1.33^{a}
Miglitol, 3 mg/kg	14.4 ± 1.7^{b}	19.6	9.7 ± 1.3^{b}	96.4 ± 18.9^{a}	3.29 ± 1.13^{a}
Sitagliptin, 0.3 mg/kg	11.5 ± 1.6^{bc}	35.8	10.0 ± 1.8^{b}	252.5 ± 86.1^{b}	4.62 ± 3.06^{a}
Combination	9.4 ± 1.3^{c}	47.5	6.2 ± 1.3^{c}	107.3 ± 20.7^{a}	11.22 ± 9.72^{b}

Maltose-loading tests used for normal mice. Enteral nutirition (EN)-loading tests used for mice fed high-fat diet for 6 weeks. Each value represents the mean ± SD of 8–10 mice. Means with different letters (a, b, and c) are significantly different at $P < 0.05$ by Tukey's multiple comparison test. $AUC_{0-2\ h}$, the area under the curve of blood glucose levels for up to 2 h after maltose-loading; GLP-1, glucagon-like peptide-1.

burden on pancreatic β-cells. In addition, a correlation between hyperinsulinemia and the level of high-sensitivity C-reactive protein (CRP), a marker of inflammation, has been reported[10]. Because the CRP elevation is related to coronary heart disease, stroke and mortality[5], insulin secretion should be reduced as much as possible. In the present study, when α-GI and sitagliptin were used in combination, insulin secretion was suppressed to a level almost the same as that with α-GI alone. Furthermore, after chronic combined treatment of miglitol and sitagliptin for 8 weeks in high-fat diet fed mice, the elevation of fasting plasma insulin level was significantly suppressed compared with that of control mice (normal diet group: 137.8 ± 84.4 pmol/L; high-fat control group: 253.1 ± 72.3 pmol/L, $P < 0.05$ *vs* normal diet group; miglitol alone group: 189.4 ± 110.2 pmol/L; sitagliptin alone group: 359.9 ± 187.7 pmol/L; combination group: 161.9 ± 84.4 pmol/L, $P < 0.05$ *vs* high fat control; Y.H. and J.T., unpublished data). Thus, combined treatment might reduce risk of the development/progression of atherosclerosis as well as dysfunction of pancreatic β-cells.

In patients with type 2 diabetes, miglitol has been reported to increase plasma active GLP-1 levels[2,11]. GLP-1 has several physiological activities, including a trophic effect on the pancreatic islets and suppression of gastric emptying and appetite[12], in addition to enhancement of insulin secretion and inhibition of glucagon secretion. When combined with sitagliptin, each α-GI tested increased the active GLP-1 concentration synergistically. The GLP-1 secretion induced by α-GI might result from delayed absorption of carbohydrate into the lower parts of the digestive tract[13], although the details of this mechanism remain unclear.

In conclusion, combined treatment with α-GI miglitol, which more strongly inhibits the early phase of postprandial hyperglycemia, and sitagliptin can yield complementary and synergistic effects and therefore might represent a better antihyperglycemic therapy.

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