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Treatment with DPP-4I Anagliptin or α-GI Miglitol Reduces IGT Development and the Expression of CVD Risk Factors in OLETF Rats

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Summary It has been reported that postprandial hyperglycemia from the pre-diabetic stage, especially from the impaired glucose tolerance (IGT) stage, is positively associated with subsequent incidences of cardiovascular diseases (CVD) and type 2 diabetes. In this study, we aimed to investigate whether treatment with a dipeptidyl peptidase-4 inhibitor (DPP-4I) or an α-glucosidase inhibitor (α-GI), either of which suppresses postprandial hyperglycemia, reduces the expression of CVD risk factors in an IGT animal model. A DPP-4I, anagliptin (1,200 ppm), or an α-GI, miglitol (600 ppm), in the diet was administered for 47 wk to Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model for spontaneously-developed type 2 diabetes, at the IGT stage. We examined whether each treatment reduced the expression of CVD risk factors such as inflammatory cytokines/cytokine-like factors in peripheral leukocytes and adhesion molecules in the aortic tissues and circulation. Treatment with either drug reduced IGT development and repressed expression of the interleukin-1β, tumor necrosis factor-α, S100a9, and S100a11 genes in peripheral leukocytes in the fasting state at weeks 25 and 39. The mRNA levels of E-selectin in aortic tissues and protein levels of the soluble forms of E-selectin and ICAM-1 in arterial blood were significantly lower in the anagliptin and miglitol groups than in the control group. Our results suggest that long-term treatment with anagliptin or miglitol in OLETF rats at the IGT stage suppresses the expression of inflammatory cytokines in peripheral leukocytes and adhesion molecules in aortic tissues.

Key Words postprandial hyperglycemia, impaired glucose tolerance, cardiovascular disease, inflammatory cytokines, adhesion molecules

Recent cohort studies such as Diabetes Epidemiology: Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) in Europe and FUNAGATA in Japan have demonstrated that postprandial hyperglycemia is a strong independent risk factor for the development of cardiovascular diseases (CVD) in subjects with impaired glucose tolerance (IGT), reflecting pre-diabetic subjects, or type 2 diabetes (*1*, *2*). Furthermore, the Study To Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) trial reported that suppression of postprandial hyperglycemia in subjects with IGT, the pre-diabetic stage, by treatment with the α-glucosidase inhibitor (α-GI) acarbose, which suppresses postprandial hyperglycemia by inhibiting the carbohydrate digestion in the small intestine, reduces the incidence of CVD as well as the development of type 2 diabetes (*3*). These lines of evidence indicate that inhibition of postprandial hyperglycemia from the pre-diabetic stage could inhibit CVD development.

Many previous studies have shown that CVDs are related to activation of leukocytes such as neutrophils, monocytes, and macrophages, and associated disorders of vascular endothelial function. Postprandial hyperglycemia activates the leukocytes through the production of reactive oxygen species (ROS) by activated neutrophils and enhanced glucose metabolism in mitochondria (*4*). The activated leukocytes secrete inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α (*5*, *6*). These inflammatory cytokines

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Abbreviations: α-GI, α-glucosidase inhibitor; ALT, alanine aminotransferase; ANOVA, analysis of variance; AST, aspartate aminotransferase; CVD, cardiovascular diseases; DPP-4, dipeptidyl peptidase-4; DPP-4I, dipeptidyl peptidase-4 inhibitor; GLP, glucagon-like peptide; γ-GTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HUVECs, human umbilical vein endothelial cells; ICAM, intercellular adhesion molecule; IFN, interferon; IGT, impaired glucose tolerance; IL, interleukin; LDL, low-density lipoprotein; NEFA, non-esterified fatty acid; OGTT, oral glucose tolerance test; OLETF rat, Otsuka Long-Evans Tokushima Fatty rat; ROS, reactive oxygen species; TG, triacylglycerol; TNF, tumor necrosis factor; STZ, streptozotocin; VCAM, vascular cell adhesion molecule.

trigger the production of E-selectin, intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1, which are adhesion molecules from the vascular endothelium (*7*). These adhesion molecules enhance the attachment of leukocytes, particularly neutrophils, to the vascular endothelium, and the leukocytes then secrete ROS and a protease, neutrophil elastase, resulting in the induction of vascular endothelium function disorders (*8*). It has been reported in several cross-sectional studies that circulating IL-1β and TNF-α concentrations are positively associated with moderate obesity, IGT, and/or type 2 diabetes mellitus in Japan and Western countries (*9–11*). In addition, previous longitudinal and cross-sectional studies including Japanese populations have demonstrated that serum concentrations of soluble (s) E-selectin in particular, as well as sICAM-1 and sVCAM-1, are positively associated with arteriosclerosis-related clinical parameters and the subsequent incidence of CVD in type 2 diabetic patients (*12–15*). These results indicate that circulating levels of inflammatory cytokines such as IL-1β and TNF-α, and soluble adhesion molecules such as sE-selectin, sICAM-1, and sVCAM-1 could be indicators for subsequent development of CVD as well as type 2 diabetes. Our previous study demonstrated that treatment with miglitol, an α-GI that effectively represses postprandial hyperglycemia earlier than other α-GIs such as acarbose and voglibose, from the pre-diabetic stage reduced the expression of inflammatory cytokine genes (IL-1β, TNF-α, IL-6, and interferon (IFN)-γ) in peripheral leukocytes of Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of natural type 2 diabetes development (*16*). Furthermore, our recent study revealed that increases in the IL-1β, TNF-α, and S100a8/9/11 mRNA levels in the peripheral leukocytes of OLETF rats at the IGT stage by a single oral sucrose loading were suppressed by administration of a dipeptidyl peptidase (DPP)-4 inhibitor (DPP-4I), anagliptin, or an α-GI, miglitol (*17*). S100 proteins are known to be diagnostic inflammatory markers for cancer, Kawasaki disease, Alzheimer disease, and diabetes, and some of them (S100a8/9/12) are also thought to be cytokine-like factors (*18*). DPP-4I treatment suppresses postprandial hyperglycemia by promoting insulin secretion through enhanced levels of the biologically active peptides incretins in the blood by inhibition of their breakdown, with a smaller risk of the incidence of hypoglycemia in type 2 diabetic patients (*19*, *20*). Thus, it appears that treatment of pre-diabetic animals with either insulin-independent drugs (α-GIs) or insulin-dependent drugs (DPP-4Is) can reduce the expression of CVD risk factors. However, it remains unknown whether long-term treatment with a DPP-4I can reduce inflammatory cytokine expression in peripheral leukocytes of animals at the IGT stage. Furthermore, it has not been examined whether DPP-4I or α-GI treatment in IGT model animals can repress the expression of adhesion molecules (E-selectin, ICAM-1, and VCAM-1) in aortic tissues and the soluble forms of these proteins in the blood.

In this study, we examined whether long-term treatment with the DPP-4I anagliptin or α-GI miglitol, either of which suppresses postprandial hyperglycemia, reduces the expression of inflammatory cytokines in peripheral leukocytes, adhesion molecules in aortic tissues, and circulating soluble adhesion molecules in OLETF rats at the pre-diabetic (IGT) stage.

MATERIALS AND METHODS

Animals. Twenty-four male OLETF rats, 4 wk of age, were obtained from the Otsuka GEN Research Institute (Tokushima, Japan). The rats were bred in individual cages and maintained at a constant temperature (23±2°C) and humidity (55±5%) under a 12-h/12-h light/dark cycle (lights on: 07:00–19:00). After being fed a standard laboratory diet (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) for 10 d, all rats consumed a 40% sucrose solution ad libitum from a water bottle during the period from 6–10 wk of age. At 4 wk after the start of sucrose solution administration, the rats were given regular water and fed a high carbohydrate experimental rodent diet (control diet) until 13 wk of age. The diet was based on the recommendations of the American Institute of Nutrition (AIN) (*21*) and contained 20% (w/w) casein, 27.8% cornstarch, 27.8% sucrose, 9.5% corn oil, 5% lard, 3.5% AIN93 vitamin mix, 1% AIN93G mineral mix, 5% cellulose, 0.3% L-cystine, and 0.25% choline bitartrate. The rats were then assigned to one of three groups (n=8) without significant differences in their 14-h-fasted plasma glucose concentrations [mean± standard error of the mean (SE): 142.7±1.8 mg/dL], hemoglobin A1c (4.8±0.1%), or body weight (453.1±1.2 g). From 13 wk of age, the control group rats were fed a control diet, the miglitol group rats were fed a diet containing 600 ppm (0.6 g/1,000 g diet) miglitol, and the anagliptin group rats were fed a diet containing 1,200 ppm (1.2 g/1,000 g diet) anagliptin. Both drugs were provided by Sanwa Kagaku Kenkyusho Co. Ltd. (Aichi, Japan), and replaced in cellulose. All rats were allowed free access to their respective diet and tap water for 47 wk. At 11, 25, and 39 wk after the start of the experimental diets, following a 14-h fast, blood samples were collected from the tail vein using a capillary tube (Terumo Co. Ltd., Tokyo, Japan). All rats were subjected to an oral glucose tolerance test (OGTT) at 45 wk after the start of the experimental diets. Plasma samples for glucose analyses and blood RNA extractions were collected from the tail vein. The rats were euthanized by decapitation in a non-fasting state at 47 wk after the start of the experimental diets (60 wk of age), and arterial blood and aortic tissues were collected. The experimental procedures used in the present study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

OGTT. An OGTT was performed in the rats at 45 wk after the start of the experimental diets. The rats were orally administered a 40% glucose solution (2 g/kg body weight) after a 14-h fast (20:00–10:00). Blood samples were collected from the tail vain using capillaries just before (0 min) and at 15, 30, 60, 90, 120, and 180 min after glucose administration for plasma and serum sam-

Table 1. Sequences of the oligonucleotide primers used for real-time RT-PCR.

Target mRNA	Sequence
Interleukin 1 beta (IL-1β) (#78[1])	5′-TGTGATGAAAGACGGCACAC-3′ 5′-CTTCTTCTTTGGGTATTGTTTGG-3′
Tumor necrosis factor (TNF-α) (#63)	5′-GTCTACTGAACTTCGGGGTGA-3′ 5′-ATGAGAGGGAGCCCATTTG-3′
S100 calcium binding protein A8 (S100a8) (#26)	5′-GCCACAAGGAGTAACAGAGCTT-3′ 5′-TGACGACTTTATTCTGTAGACATATCC-3′
S100 calcium binding protein A9 (S100a9) (#85)	5′-AAGGACTTGCCAAATTTTCTGA-3′ 5′-GGACAGTTGATTGTCCTGGTTT-3′
S100 calcium binding protein A11 (S100a11) (#44)	5′-CGACCGCATGATGAAGAAG-3′ 5′-AAGCCACCAATAAGGTTGAGAA-3′
Intercellular Adhesion Molecule 1 (ICAM-1) (#29)	5′-GCAGACCACTGTGCTTTGAG-3′ 5′-TCCAGCTCCACTCGCTCT-3′
Vascular cell adhesion molecule 1 (VCAM-1) (#13)	5′-CAAATGGAGTCTGAACCCAAA-3′ 5′-GGTTCTTTCGGAGCAACG-3′
Selectin E (E-selectin) (#18)	5′-TCTAAGATGCGAGCAATCAGG-3′ 5′-CAGGATTCTGCAGACAGTTCA-3′
TATA-box binding protein (TBP) (#129)	5′-CCCACCAGCAGTTCAGTAGC-3′ 5′-CAATTCTGGGTTTGATCATTCTG-3′

[1] The numbers in parentheses indicate the IDs of the universal probes (Roche Diagnostics, Tokyo, Japan) used to detect the signals for the individual genes.

ples, and at 0 and 180 min for RNA extraction samples.

Blood collection. Blood samples from the tail vein for plasma, RNA extraction, and serum were collected using a capillary treated with sodium fluoride and heparin-lithium, a capillary treated with heparin-lithium, and a plain capillary (Terumo Co. Ltd.), respectively. Blood samples for serum from the carotid artery at decapitation (60 wk of age) were collected using a Veneject II blood collection tube (Terumo Co. Ltd.). The blood samples for plasma and serum were centrifuged at 1,000 $\times g$ at 4°C for 20 min, and the supernatants were stored at −80°C until analysis. The blood samples for RNA extraction were immediately placed in 500 μL of PAXgene RNA fixation solution (Qiagen/BD, Venlo, Netherlands) to fix the leukocytes without changing their mRNA levels. The fixation solution containing the blood samples was incubated at room temperature for 24 h, and then stored at −80°C according to the manufacturer's instructions.

Blood biochemical parameters. Glucose concentrations in plasma at weeks 0, 11, 25, and 39 and those in serum from the carotid artery at week 47 of the experimental period were determined using a Glucose C-II-test (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Serum insulin concentrations were determined using a Rat Insulin ELISA Kit (AKRIN-010; Shibayagi, Gunma, Japan). Triacylglycerol (TG), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, non-esterified fatty acid (NEFA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) concentrations were measured using commercial kits (TG, TG L-type Wako; LDL-cholesterol, LDL L-type Wako; HDL-cholesterol, HDL L-type Wako; NEFA, NEFA-HA2 test Wako; AST, AST L-type Wako; ALT, ALT L-type Wako; γ-GTP, γ-GTP L-type Wako; Wako Pure Chemical Industries).

RNA extraction and real-time RT-PCR. Total RNA from the fixed blood samples was extracted using an RNeasy Kit (Qiagen), and regarded as total RNA from peripheral leukocytes because mature red blood cells have few total RNAs. The aortic tissues were homogenized in a solution consisting of 4 M guanidine thiocyanate, 25 mM sodium citrate (pH 7.5), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol, and stored at −80°C until RNA extraction. Total RNA was extracted from the homogenates using an acidified guanidine thiocyanate method described by Chomczynski and Sacchi (*22*). Total RNA samples isolated from peripheral leukocytes (100 ng) and aortic tissues (240 ng) were converted to cDNA by reverse transcription using SuperScript™ III RT (Invitrogen) according to the manufacturer's instructions. To quantitatively estimate the mRNA levels, PCR amplification was performed using a LightCycler 480 instrument (Roche Molecular Biochemicals, Mannheim, Germany).

Table 2. Body weights and fasting blood glucose concentrations in the OLETF rats assigned to each experimental group.

		week 0	Week 11	Week 25	Week 39
Body weight (g)	Control	447±16	719±21	905±33	1,033±48
	Miglitol	455±6	688±19	852±27	975±41
	Anagliptin	449±13	710±23	888±30	948±39
Fasting blood glucose (mg/dL)	Control	144±13	140±5	179±4	164±7
	Miglitol	126±6	130±4	161±10	156±13
	Anagliptin	153±7	150±4	182±8	175±11

Values are expressed as means±SE for 6–7 rats.

Table 3. Basic clinical parameters in the OLETF rats assigned to each experimental group.

	Control	Miglitol	Anagliptin
Body weight (g)	1,087±76	1,030±48	977±37
Liver weight (g)	27.8±2.1	29.9±0.7	27.8±1.1
Mesenteric adipose tissue weight (g)	16.2±1.3	16.5±0.4	17.1±0.8
Epididymal adipose tissue weight (g)	28.2±3.6	29.7±1.9	29.0±1.9
Kidney weight (g)	4.3±0.4	5.1±0.3	4.4±0.3
Glucose (mg/dL)	279±46	229±11	232±10
TG (mg/dL)	237±78	179±28	143±14
LDL-cholesterol (mg/dL)	81±17	100±7	79±8
HDL-cholesterol (mg/dL)	120±22	148±8	135±12
NEFA (mEq/L)	0.59±0.05	0.55±0.04	0.49±0.06
AST (IU/L)	100±15	68±12	77±10
ALT (IU/L)	41±5[a]	28±2[b]	33±2[ab]
γ-GTP (IU/L)	4.0±2.4	1.5±0.1	1.5±0.1

Values are expressed as means±SE for 6–7 rats.
[a,b] Values not sharing a common letter differ significantly from each other at $p<0.05$ by Tukey's test based on one-way ANOVA.
TG, triacylglycerol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; NEFA, non-esterified fatty acid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase.

Real-time RT-PCR was carried out in a total volume of 10 μL containing each of gene-specific primers (Rikaken, Aichi, Japan), cDNA, and LightCycler 480 Probes Master (Roche Molecular Biochemicals). Real-time RT-PCR amplification of the cDNA from the peripheral leukocytes and aortic tissues was performed using a universal probe library system of the LightCycler instrument. The amplification conditions were as follows: activation of Taq DNA polymerase and denaturation of cDNA at 95°C for 5 min; and a three-step PCR program, comprising 50 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 25 s, and extension at 72°C for 1 s. The cycle threshold (CT) values of each gene detected by real-time RT-PCR were converted into signal intensities by the delta-delta CT method (*23*), which calculates the difference of one CT value as a 2-fold difference between the signals of each gene and an internal control gene (TBP) using the formula

$[2^{(\text{CT of TBP}-\text{CT of each gene})}]$.

The PCR primer sequences for the rat peripheral leukocytes and aortic tissues are listed in Table 1.

Multiplex immunoassay for protein levels of adhesion molecules in serum. The serum concentrations of sICAM-1 and sE-selectin proteins were measured using a MILLIPLEX™ MAP Kit Rat Cardiovascular Disease (CVD) Panel 2 (Cat# RCVD2-89K; Millipore, Billerica, MA) according to the manufacturer's instructions. Signals were detected by a Luminex instrument (Luminex Corporation, Austin, TX).

Statistical analysis. All data were expressed as means±SE. The significance of differences among groups was determined by Tukey's test based on one-way analysis of variance (ANOVA) or paired two-way ANOVA. Values of $p<0.05$ were considered to indicate statistical significance.

RESULTS

Effects of treatment with anagliptin or miglitol on body and tissue weights, and blood glucose concentrations of OLETF rats

The body weights at weeks 0, 11, 25, and 39 after the start of feeding the experimental diets are shown in Table 2. The body weights did not differ significantly among the control group and the two groups receiving the diets containing miglitol or anagliptin at any of the time points examined (Table 2). Other basic clini-

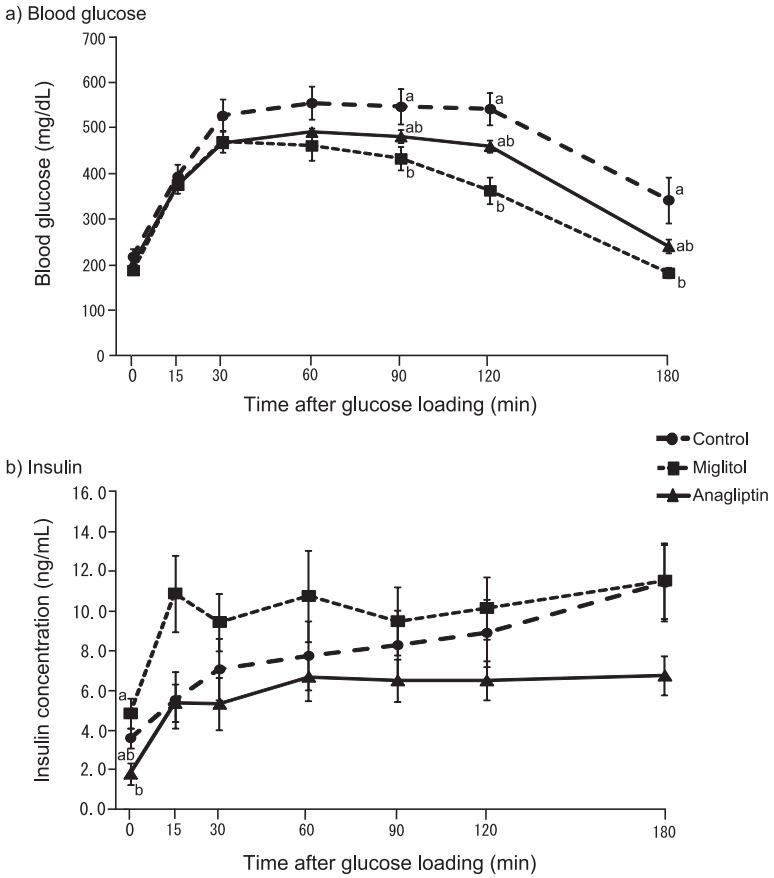

Fig. 1. Effects of oral glucose loading on the plasma glucose and serum insulin concentrations in the OLETF rats assigned to each experimental group. (a) Plasma glucose concentration. (b) Serum insulin concentration. The data are means±SE for 6–7 animals. Values not sharing a common letter differ significantly from each other among the treatment groups by Tukey's test based on one-way ANOVA.

cal parameters also showed no differences at any of the time points examined. The glucose concentrations in blood collected in the 14-h fasting state did not differ among the three groups, and neither did the levels in serum obtained from the aorta in the non-fasting state at week 47 of feeding the experimental diets. The body, adipose tissue, and kidney weights at week 47 of feeding the experimental diets did not differ among the groups. The ALT activity was significantly lower in the miglitol group than in the control group (Table 3).

The plasma concentrations of glucose and insulin in the OGTTs at week 45 of feeding the experimental diets are shown in Fig. 1. In the control group, the plasma glucose concentration after oral glucose loading reached a peak at 60 min (555±36 mg/dL), and then remained at a high level until 180 min (342±50 mg/dL). The plasma glucose concentrations at 60–180 min tended to be lower in both the miglitol and anagliptin groups compared with the control group, and were significantly lower in the miglitol group than in the control group at 90–180 min. The area under the curve (AUC) values for the glucose concentrations in the OGTT in the control, miglitol, and anagliptin groups were 47,964±3,496, 32,489±3,171, and 38,260±1,347, respectively. The AUC was significantly higher in the control group than in the miglitol group ($p<0.05$). The AUC tended to be lower in the anagliptin group than in the control group, but the difference was not significant ($p=0.085$). The AUC values for the insulin concentrations in the control, miglitol, and anagliptin groups were 844.8±222.6, 972.0±190.8, and 782.5±190.0, respectively. These values did not differ significantly among the groups. The insulin concentration in the fasting state was significantly lower in the anagliptin group than in the miglitol group.

Effects of treatment with anagliptin or miglitol on the expression of genes related to inflammation in the peripheral leukocytes of OLETF rats

In the control group, the mRNA levels of IL-1β, TNF-α, S100a9, and S100a11 in the peripheral leukocytes were significantly higher at week 39 than at week 0. In contrast, no significant differences in the mRNA levels of these genes were observed between week 0 and week 39 in the animals fed a diet containing miglitol or anagliptin. The mRNA levels of TNF-α in the peripheral leukocytes at weeks 11 and 25 were significantly higher than those at the start of the feeding period, and the enhanced TNF-α mRNA levels were significantly higher in the control group than in the miglitol and anagliptin groups at the respective time points. The mRNA levels of S100a11 in the peripheral leukocytes tended to be lower in the rats fed a diet containing miglitol or anagliptin,

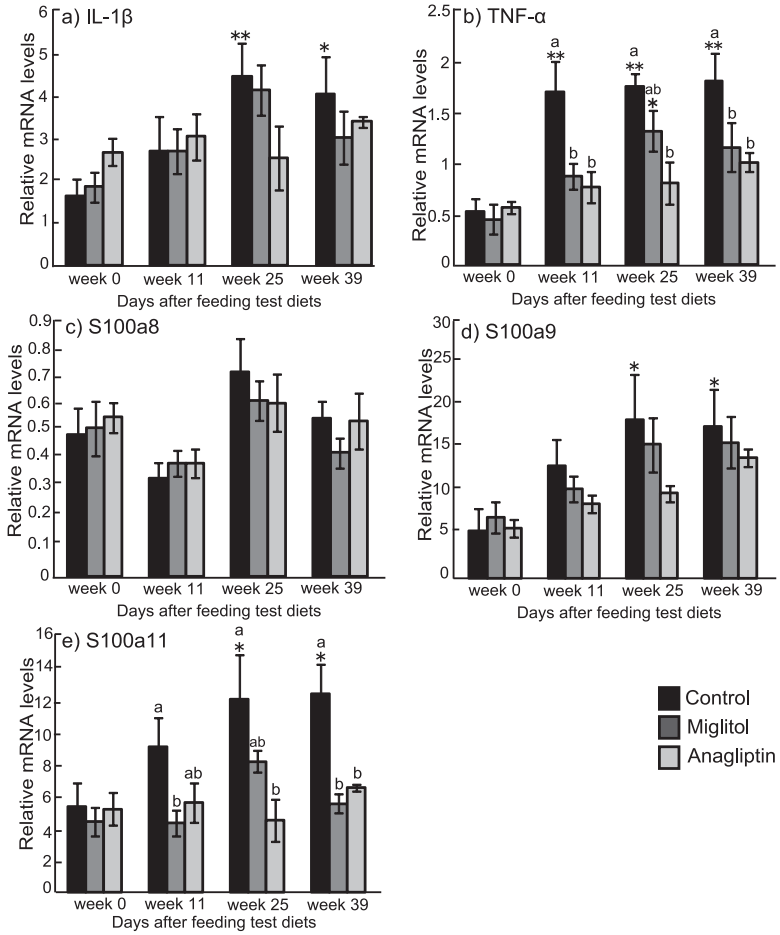

Fig. 2. Effects of treatment with anagliptin or miglitol for 47 wk on the expression of inflammation-related genes in peripheral leukocytes of OLETF rats. The data are means±SE for 6–7 animals. Values not sharing a common letter differ significantly from each other among the treatment groups at the same week or differ significantly from each other among the treatment groups by Tukey's test based on repeated two-way ANOVA. $^{*}p<0.05$, $^{**}p<0.01$, significant differences compared with week 0 (Tukey's test based on repeated two-way ANOVA).

with significant differences in the S100a11 mRNA levels observed between the control and miglitol groups at weeks 11 and 39, and between the control and anagliptin groups at weeks 25 and 39 (Fig. 2).

Effects of treatment with miglitol or anagliptin on the expression of adhesion molecules in the aortic tissues and blood of OLETF rats

The mRNA levels of VCAM-1 in the aortic tissues did not differ significantly among the three groups. The mRNA levels of ICAM-1 were not significantly reduced by anagliptin. However, the mRNA levels of E-selectin were significantly lower in the miglitol and anagliptin groups than in the control group. Next, we examined the circulating protein levels of sE-selectin and sICAM-1. The protein levels of sE-selectin and sICAM-1 in the arterial blood were significantly lower in the miglitol and anagliptin groups than in the control group (Fig. 3).

DISCUSSION

Our previous study demonstrated that miglitol or anagliptin treatment in OLETF rats reduced postprandial hyperglycemia in sucrose-loading tests (*17*). In this study, we demonstrated that long-term treatment with these drugs reduced (miglitol) or tended to reduce (anagliptin) IGT development in OLETF rats. It is known that miglitol represses postprandial glucose and insulin levels by inhibiting α-glucosidases in the small intestine (*24*). Generally, suppression of insulin secretion by inhibition of postprandial hyperglycemia protects against apoptosis of pancreatic β-cells and maintains the insulin secretion capacity. Indeed, we reported that long-term treatment with miglitol in OLETF rats maintained the capacity of insulin secretion and reduced the development of type 2 diabetes (*25*). These findings indicate that miglitol has potency to protect the insulin secretion capacity of pancreatic β-cells by reducing postprandial insulin secretion. However, in the present study, we did not find significant protection of the insulin secretion capacity in OLETF rats by miglitol treatment. The reasons for the differences between the present and previous studies may be differences in the diet compositions (sucrose and fat were higher and starch was lower in the present study than in the previous study) and the treatment periods with miglitol (47 wk in the present study versus 65 wk in the previous study). In the present study, we found that glucose clearance from 90 to 180 min in the

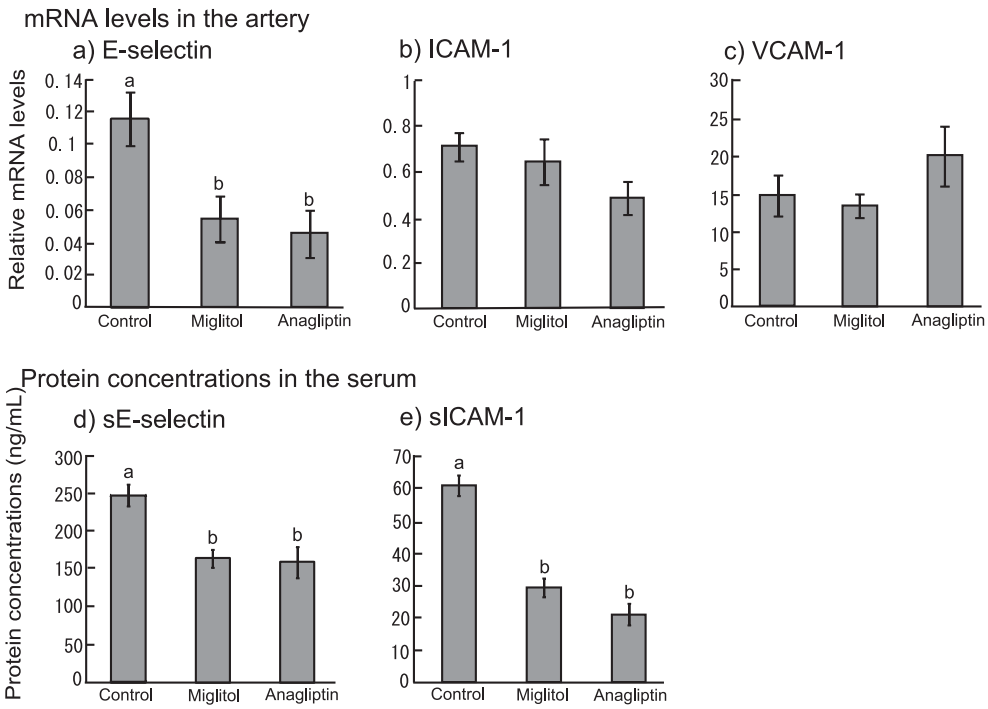

Fig. 3. Effects of treatment with miglitol or anagliptin on the expression of adhesion molecules in aortic tissues and arterial blood of OLETF rats. (a–c) mRNA levels in the artery. (d, e) Protein concentrations in the serum. The results of quantitative RT-PCR analyses for individual samples were normalized by the corresponding TBP mRNA abundance. The data are means±SE for 5–7 animals. Values not sharing a common letter differ significantly from each other among the treatment groups by Tukey's test based on one-way ANOVA.

OGTT was lower in the miglitol group than in the control group. These results indicate that miglitol treatment may reduce insulin resistance in OLETF rats. Regarding anagliptin, the AUC values for the glucose concentrations in the OGTT tended to be lower in the anagliptin group than in the control group ($p=0.085$). However, the AUC values for the insulin concentrations did not differ between the anagliptin and control groups, and the fasting insulin concentrations were lower in the anagliptin group than in the miglitol group. Thus, anagliptin treatment may improve insulin resistance in OLETF rats. Indeed, previous studies showed that another DPP-4 inhibitor, teneligliptin, improved insulin resistance in type 2 diabetic patients (*26*) and that anagliptin ameliorated insulin resistance in the mouse liver (*27*). The issue of whether treatment with miglitol or anagliptin ameliorates insulin resistance in OLETF rats should be further assessed using insulin tolerance tests.

In this study, we have demonstrated that expression of the IL-1β, TNF-α, S100a9, and S100a11 genes in peripheral leukocytes increased during IGT development and that treatment with either anagliptin or miglitol in OLETF rats at the IGT stage reduced the induction of these genes in peripheral leukocytes in the fasting state. Furthermore, we found that not only the mRNA level of E-selectin in aortic tissues, but also the serum protein concentrations of sE-selectin and sICAM-1, were reduced by treatment with either miglitol or anagliptin in OLETF rats. E-selectin on the surface of the vascular endothelium provokes "rolling of leukocytes," which is the phenomenon of leukocytes rolling over around the vascular endothelium through binding of E-selectin in the vascular endothelium to the selectin ligand in the surface of leukocytes (*28*). This is the initial step of leukocyte infiltration, and the leukocytes subsequently bind closely to the vascular endothelium through ICAM-1 and VCAM-1 (*29*, *30*). It should be noted that the circulating sICAM-1 protein levels, but not the mRNA levels, in aortic tissues were reduced by treatment with either miglitol or anagliptin in OLETF rats. A recent study reported that the ICAM-1 protein level was regulated by protein degradation in the proteasome and lysosome in the intestinal cell line Caco-2 (*31*), although it has not been reported in endothelial cells or the aortic tissues of animals. These results indicate that the sICAM-1 protein level may be regulated at the protein degradation and/or protein synthesis levels. These aspects need to be examined in further studies. Taking the results of this study together with evidence from previous studies, inhibition of postprandial hyperglycemia by either the DPP-4I anagliptin or α-GI miglitol from the IGT stage reduce expressions of inflammatory cytokine/cytokine-like factor genes in peripheral leukocytes and adhesion molecules, in particular E-selectin, in the aortic tissues and circulation.

The present results indicate that inhibition of postprandial hyperglycemia from the pre-diabetic stage is important for preventing CVD development. This issue is associated with human intervention studies such as the STOP-NIDDM (*3*). In addition, many cohort stud-

ies have demonstrated that consumption of foods with high glycemic indexes is positively associated with the subsequent incidence of CVD in type 2 diabetic patients (*32*). It was reported that interventions for obese women involving caloric restriction (by 500–1,000 kcal/d) and consumption of 50 g/d of a fiber supplement for 12 wk reduced the plasma levels of IL-6 and IL-18 (*33*). Interventions for overweight and obese adults by a low-glycemic load diet for 28 d reduced the serum levels of C-reactive protein, a typical clinical inflammation marker (*34*). Thus, interventions with these dietary lifestyle factors may reduce inflammatory cytokine/cytokine-like factor gene expressions in peripheral leukocytes and adhesion molecules in the aortic tissues and circulation and the associated CVD in animals and humans. However, these issues should be examined in further studies.

In this study, we used miglitol and anagliptin at concentrations of 600 ppm and 1,200 ppm, respectively, in the diet. The mean applied doses per day were 15.2±0.4 mg for miglitol and 30.0±1.1 mg for anagliptin for individual OLETF rats. In humans, the applied doses of miglitol and anagliptin per day are 75–225 mg and 200 mg for type 2 diabetic patients, respectively. The applied dose of miglitol is determined on the basis of doses with lower incidence of digestive symptoms such as diarrhea and with potency for reducing postprandial hyperglycemia. Previous studies have demonstrated that doses of 400 and 800 ppm in the diet did not cause the incidence of digestive symptoms in rats (*25*, *35*). In this study, we did not observe digestive symptoms in the OLETF rats fed a diet containing 600 ppm miglitol. Regarding anagliptin, feeding diets containing 500 and 3,000 ppm anagliptin to mice for 10 or 16 wk reduced IGT development in a dose-dependent manner (*27*, *36*). Therefore, it is considered that the doses of miglitol and anagliptin in the diet used in this study are within adequate ranges for OLETF rats.

It should be noted that we did not examine the morphological changes that occurred in the aortic tissues. The reason why we did not examine these changes is that rodents do not generally develop atherosclerosis. To investigate the effects of drugs and food factors on atherosclerosis development, many scientists use the animal model of ApoE-deficient mice. However, these model mice do not exhibit the development and progression of type 2 diabetes. Thus, we assessed the putative risks of CVD development by measuring inflammatory cytokine gene expression in peripheral leukocytes and circulating soluble adhesion molecules that were related to the development of type 2 diabetes and/or CVD in human studies (*9*–*15*). In this study, we found reductions in inflammatory cytokines/cytokine-like factors such as TNF-α and S100a11 gene expression in peripheral leukocytes and adhesion molecules such as sE-selectin and sICAM-1 in arterial blood after anagliptin or miglitol treatment. However, it remains necessary to examine whether anagliptin or miglitol can suppress the attachment of macrophages to the aortic tissues and atherosclerosis development in ApoE-deficient mice with IGT by crossing them with type 2 diabetic models such as *ob*/*ob* mice.

In conclusion, the results of this study indicate that long-term treatment with the DPP-4I anagliptin or α-GI miglitol in OLETF rats reduces IGT development and represses inflammatory cytokines in peripheral leukocytes and adhesion molecules in the aortic tissues. Further studies are needed to examine whether reductions in postprandial hyperglycemia induced by dietary lifestyle factors can repress these expressions in animals and humans with IGT.

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