Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

ORIGINAL ARTICLE
Vascular Biology and Vascular Medicine

Dipeptidyl Peptidase-4 Inhibitor, Sitagliptin, Improves Endothelial Dysfunction in Association With Its Anti-Inflammatory Effects in Patients With Coronary Artery Disease and Uncontrolled Diabetes

Junichi Matsubara, MD, PhD; Seigo Sugiyama, MD, PhD; Eiichi Akiyama, MD; Satomi Iwashita; Hirofumi Kurokawa, MD; Keisuke Ohba, MD; Hirofumi Maeda, MD; Koichiro Fujisue, MD; Eiichiro Yamamoto, MD; Koichi Kaikita, MD, PhD; Seiji Hokimoto, MD, PhD; Hideaki Jinnouchi, MD, PhD; Hisao Ogawa, MD, PhD

Background: Dipeptidyl peptidase 4 (DPP4) inhibitors are used for treatment of diabetes mellitus (DM). We hypothesized that sitagliptin, a DPP4-inhibitor, could improve endothelial dysfunction in DM patients with coronary artery disease (CAD).

Methods and Results: The 40 patients with CAD and uncontrolled DM, aged 68.7±9.4 years (mean±standard deviation) (50% males, hemoglobin A_{1c} [HbA_{1c}] 7.4±1.0%) were assigned to either additional treatment with sitagliptin (50 mg/day, n=20) or aggressive conventional treatment (control, n=20) for 6 months. Endothelial function was assessed by the reactive hyperemia peripheral arterial tonometry index (RHI). The clinical characteristics at baseline were not different between the groups. After treatment, fasting blood glucose and insulin levels, and lipid profiles were not different between the groups. HbA_{1c} levels significantly improved similarly in both groups. The percent change in RHI was greater in the sitagliptin group than in the control group (62.4±59.2% vs. 15.9±22.0%, P<0.01). Furthermore, treatment with sitagliptin resulted in a significant decrease in the high-sensitivity C-reactive protein (hsCRP) level, but no such change was noted in the control group. Linear regression analysis demonstrated a significant negative relation between changes in RHI and hsCRP, but not between RHI and HbA_{1c}.

Conclusions: Sitagliptin significantly improved endothelial function and inflammatory state in patients with CAD and uncontrolled DM, beyond its hypoglycemic action. These findings suggest that sitagliptin has beneficial effects on the cardiovascular system in DM patients. (*Circ J* 2013; **77:** 1337–1344)

Key Words: Dipeptidyl peptidase-4 inhibitors; Endothelial function; Inflammation

Diabetes mellitus (DM) increases the risk of atherosclerosis and cardiovascular disease, and is also known to worsen the outcome after surviving a cardiovascular (CV) event.[1] However, glucose-lowering medications do not provide enough cardiovascular protection in DM patients and whether they reduce CV events remains controversial.[2]

Editorial p1156

Atherosclerosis is an inflammatory disease of the arterial wall and accumulating evidence suggests the involvement of endothelial dysfunction in all stages of atherogenesis.[3,4] Approaches designed to reduce the inflammatory activity and improve endothelial function may therefore have additional therapeutic value in the prevention and treatment of atherosclerotic diseases, including coronary artery disease (CAD).[5]

The new class of anti-type-2 DM drugs, including dipeptidyl peptidase-4 (DPP4) inhibitors and glucagon-like peptide 1 (GLP-1) analogs, improve glucose metabolism through the activation of GLP-1 receptor signaling, which induces insulin secretion and suppresses glucagon secretion in the pancreas.[6,7] Several studies have reported the beneficial effects of GLP-1

Received September 18, 2012; revised manuscript received December 12, 2012; accepted January 10, 2013; released online February 2, 2013 Time for primary review: 13 days

Department of Cardiovascular Medicine, Faculty of Life Sciences, Graduate School of Medical Sciences, Kumamoto University, Kumamoto (J.M., S.S., E.A., S.I., H.K., K.O., H.M., K.F., E.Y., K.K., S.H., H.O.); Department of Cardiovascular Clinical and Translational Research (J.M.), Department of Interventional Cardiology (S.H.), Department of Preventive Cardiology (H.J.), Kumamoto University Hospital, Kumamoto; and Jinnouchi Hospital, Kumamoto (H.J.), Japan

Mailing address: Seigo Sugiyama, MD, PhD, Department of Cardiovascular Medicine, Faculty of Life Sciences, Graduate School of Medical Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan. E-mail: ssugiyam@kumamoto-u.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-12-1168

Table 1. Baseline Clinical Characteristic of the Patients With CAD and Uncontrolled DM

	Sitagliptin group (n=20)	Control group (n=20)	P value
Age, years	69.9±7.6	67.5±10.9	0.43
M/F	10/10	10/10	0.99
Current smoking, n (%)	4 (20.0)	4 (20.0)	0.99
Hypertension, n (%)	14 (80.0)	16 (70.0)	0.72
Duration of DM, years	6.8 (3.0–10.0)	7.2 (1.4–14.5)	0.91
HbA_{1c}, (%)	7.3±0.8	7.5±1.2	0.40
Dyslipidemia, n (%)	11 (55.0)	13 (65.0)	0.75
History of OMI	8 (40.0)	6 (30.0)	0.74
1/2/3 vessels/LMT lesion	7/8/4/1	9/5/5/1	0.79
Gensini score	36.8 (17.0–54.8)	34.5 (27.5–67.8)	0.46
Medications			
β-blockers, n (%)	14 (70.0)	17 (85.0)	0.45
ACEIs or ARBs, n (%)	13 (65.0)	16 (80.0)	0.48
CCBs, n (%)	13 (65.0)	14 (70.0)	0.99
Nitrite, n (%)	3 (15.0)	2 (10.0)	0.99
Statins, n (%)	19 (95.0)	19 (95.0)	0.99
Antiplatelet drugs, n (%)	19 (95.0)	20 (100)	0.99
Insulin, n (%)	0 (0.0)	5 (25.0)	0.06
Sulfonylurea, n (%)	12 (60.0)	9 (45.0)	0.53
Biguanide, n (%)	7 (35.0)	4 (20.0)	0.48
α-Glucosidase inhibitors, n (%)	9 (45.0)	6 (30.0)	0.52
Pioglitazone, n (%)	3 (15.0)	3 (15.0)	0.99

Data are mean±SD, n (%) or median and (interquartile range).
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CAD, coronary artery disease; CCB, calcium-channel blocker; DM, diabetes mellitus; HbA_{1c}, hemoglobin A_{1c}; LMT, left main trunk; OMI, old myocardial infarction.

on the cardiovascular system, and exogenous GLP-1 has improved endothelial dysfunction in Dahl salt-sensitive rats and in type-2 DM patients with CAD without affecting insulin resistance.[8,9] In contrast, there is a lack of evidence for the beneficial effects of DPP4 inhibitors on the cardiovascular system. Recently, we showed that a DPP4 inhibitor significantly improved endothelial function in an atherosclerosis mouse model by augmenting endogenous GLP-1 activity in association with its anti-inflammatory effects.[10] These findings indicate that DPP4 inhibitors could have beneficial effects on the endothelium, beyond their hypoglycemic action, in DM patients.

The present study tested the hypothesis that DPP4 inhibitors would improve endothelial dysfunction in association with improvement of inflammation in DM patients with CAD. For this purpose, we investigated whether additional treatment with DPP4 inhibitor, sitagliptin, improved endothelial dysfunction in patients with CAD and uncontrolled DM.

Methods

Study Subjects

For this study, we recruited 40 patients with CAD and uncontrolled type-2 DM and endothelial dysfunction (age: 68.7±9.4 years; males 50%; hemoglobin A_{1c} [HbA_{1c}]: 7.4±1.0%, mean± standard deviation [SD]) referred to Kumamoto University Hospital, Japan. We excluded patients receiving GLP-1 receptor analog (exenatide and liraglutide) and those requiring rapid intensive glucose control. Other exclusion criteria were heart failure (New York Heart Association functional class II–IV), active systemic inflammatory disease, chronic renal failure requiring hemodialysis, active hepatic disease, collagen disease, malignancy, and acute coronary syndrome within the 3 months preceding admission. CAD was defined as patients with angiographically documented organic coronary stenosis >50% by quantitative coronary angiography (CAG) of the major coronary arteries. We enrolled the 40 patients in a stable state within 7 days after CAG. The patients were prospectively assigned to additional treatment with sitagliptin (50 mg/day, n=20) or intensification of conventional treatment (control, n=20) for 6 months. All patients were educated by hospital nutritionists and treated in accordance with the guideline of the Japan Diabetes Society. Based on the CAG results, we calculated the Gensini score as the index of the severity of coronary atherosclerosis.[11]

The study was approved by the institutional ethics review committee and signed informed consent was given by each patient before participation. This study was registered under the UMIN protocol registration system (ID UMIN000008806).

Assessment of Endothelial Function

Peripheral endothelial function was assessed by reactive hyperemia peripheral arterial tonometry (RH-PAT) using an EndoPAT2000 (Itamar Medical, Caesarea, Israel) as described previously.[12] RH-PAT measurements are largely operator-independent and a computerized algorithm with an on-line system automatically calculates the RH-PAT index (RHI); thus, there is minimal inter- or intraoperator variability. RH-PAT studies were performed when patients were in a stable, fasted state in the early morning, and before taking any medications. As described previously,[12] a blood pressure (BP) cuff was placed on the study arm, while the opposite arm served as a control. After a 10-min acclimatization period in a temperature- and light-controlled room, the baseline pulse amplitude

Table 2. Additional or Increase in Drugs

	Sitagliptin group (n=20)	Control group (n=20)	P value
β-blockers, n (%)	1 (5.0)	1 (5.0)	0.99
ACEIs or ARBs, n (%)	3 (15.0)	5 (25.0)	0.69
CCBs, n (%)	0 (0)	1 (5.0)	0.99
Nitrite, n (%)	0 (0)	0 (0)	0.99
Statins, n (%)	2 (10.0)	1 (5.0)	0.99
Antiplatelet drugs, n (%)	0 (0)	0 (0)	0.99
Insulin, n (%)	0 (0)	4 (20.0)	0.11
Sulfonylurea, n (%)	0 (0)	4 (20.0)	0.11
Biguanide, n (%)	2 (10.0)	4 (20.0)	0.66
α-Glucosidase inhibitors, n (%)	0 (0)	2 (10.0)	0.49
Pioglitazone, n (%)	0 (0)	2 (10.0)	0.49

Data are n (%). Abbreviations as in Table 1.

was measured from each fingertip for 5 min. The cuff was inflated to 60 mmHg above systolic BP or 200 mmHg for 5 min. After cuff deflation, PAT recording was continued for 10 min. The RH-PAT data were automatically analyzed in real time in an operator-independent manner (Endo-PAT2000 software, version 3.0.4). The RH-PAT value that reflected the extent of RH was calculated as the ratio of the average pulse amplitude of PAT signal over 1-min time intervals starting 1.5 min after cuff deflation to the average pulse amplitude of PAT signal over the 2.5-min time period before cuff inflation (baseline). We used natural logarithmic transformation of the RH-PAT value to calculate the RHI as:

$$\text{RHI} = \text{Ln}([\text{RH-PAT value}] \times [0.226 \times \text{Ln}(\text{baseline}) - 0.2]).$$ [12,13]

RH-PAT values were assessed at baseline and at 6 months. Endothelial dysfunction were defined as RHI <0.560. Previous studies have demonstrated that the RH-PAT technology has excellent reproducibility.[14,15]

Biochemical Measurements

In the early morning following an overnight fast, blood samples were obtained from all patients for biochemical analysis at baseline and at 6 months. Renal function was determined from the estimated glomerular filtration rate ($ml \cdot min^{-1} \cdot 1.73 m^{-2}$), which was calculated using the equation recommended by the Japanese Society of Nephrology.[16] We calculated both the homeostasis model assessment for insulin resistance (HOMA-IR = fasting blood glucose × insulin / 405), and HOMA beta cell function (HOMA-β = 360 × fasting insulin / (fasting blood glucose − 63)].

Statistical Analysis

Normally distributed parameters are expressed as mean ± SD values. The values for triglycerides, fasting insulin, HOMA-IR, HOMA-β, high-sensitivity C-reactive protein (hsCRP), and B-type natriuretic peptide were not normally distributed. Thus, the results were expressed as the median value (interquartile range) and log transformed before linear regression analysis. Differences between continuous variables were analyzed by the paired t-test, unpaired t-test, Mann-Whitney U-test, and Wilcoxon's matched pairs test as appropriate. Comparisons of categorical variables were analyzed by the chi-square test. We performed linear regression analysis to determine the association between RHI values and Gensini scores. Univariate and multivariate logistic regression analyses were used to assess the clinical markers and medications correlated with the greatest improvement of endothelial dysfunction (ΔRHI: after 6 months RHI–baseline RHI: over median value). A P value <0.05 was considered significant. All analyses were performed using the Statistical Package for Social Sciences, ver. 17.0J for Windows (SPSS Japan Inc, Tokyo, Japan).

Results

Table 1 details the baseline clinical characteristics of the study subjects, which were not significantly different between the sitagliptin and control groups. Furthermore, no significant difference was observed between the two groups for the treatment of DM. Neither the additional nor increase in drugs significantly differed between the 2 groups (Table 2). After the 6-month treatment, there were no significant differences between the groups in the fasting blood glucose and insulin levels or in the lipid profiles, although systolic BP in the sitagliptin group was lower than that in the control group (Table 3). Furthermore, the levels of HbA_{1c} significantly decreased in both groups (sitagliptin group: –0.64±0.82%, P<0.01, control group: –0.65±0.68%, P<0.001), but the changes in HbA_{1c} were the same degree of magnitude (P=0.95; Figure 1). The treatments significantly improved the RHI values in both groups (sitagliptin: 0.388±0.110 to 0.609±0.236; control: 0.428±0.096 to 0.487±0.115; Figures 2A,B). The percent change in RHI in the sitagliptin group was significantly greater than in the control group (62.4±59.2% vs. 15.9±22.0%; Figure 2C). The percent changes in RHI were not significantly different among the severity of CAD in each group (1-vessel disease [VD], 2-VD, 3-VD; control group: 19.6±7.5%, 13.2±7.7%, 24.5±9.9%, P=0.24, sitagliptin group: 62.9±7.6%, 60.3±48.1%, 65.5±78.2%, P=0.99). The Gensini scores were not significantly different between the control and sitagliptin groups (control: 34.5 [27.5–67.8]; sitagliptin: 36.8 [17.0–54.8], P=0.46). The RHI values at baseline did not significantly correlate with Gensini score in the present study (r=–0.123, P=0.45). We did not have detailed data of each patient's nutritional status in the present study. In the control group, the percent changes in RHI were not significantly different among the patients with or without angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin II receptor blockers (ARBs) (14.9±30.6% vs. 16.2±20.0%, P=0.92). In the sitagliptin group, the percent changes in RHI were not significantly different among the patients with or without ACEIs or ARBs (50.7±56.1% vs. 64.5±64.2%, P=0.72). Statin was added to 1 patient in the control group and 2 patients in the sitagliptin group during the follow-up period. In

Table 3. Comparison of Clinical and Biochemical Parameters at Baseline and 6 Months

	Sitagliptin group		Control group	
	Baseline	6 months	Baseline	6 months
SBP, mmHg	123.3±13.2	115.6±10.0*	125.4±14.5	128.6±12.1
DBP, mmHg	70.6±11.1	67.6±11.6	68.7±10.5	66.6±9.2
BMI, kg/m^2	24.2±3.6	23.7±3.3	24.2±2.7	24.3±2.9
WC, cm	91.0±10.3	89.2±8.9	90.9±8.5	91.7±6.3
Fasting glucose, mg/dl	127.0±31.2	124.1±23.8	129.2±44.9	126.9±21.1
Fasting insulin, μU/ml	7.50 (4.55–11.35)	7.50 (5.00–12.20)	5.80 (4.53–13.9)	8.20 (3.70–9.40)
HOMA-IR	2.52 (1.11–3.41)	2.27 (1.46–4.05)	2.13 (1.17–4.09)	2.25 (1.26–3.00)
HOMA-β	46.0 (32.3–72.1)	61.4 (24.8–81.8)	34.3 (24.3–72.2)	45.7 (22.4–65.0)
TC, mg/dl	149.4±25.0	140.4±29.7	159.0±25.9	142.4±22.7†
HDL-cholesterol, mg/dl	47.4±14.1	48.6±14.5	47.1±14.0	49.4±12.2
TG, mg/dl	108.0 (86.0–140.0)	103.5 (77.0–121.0)	116.5 (94.5–151.0)	121.0 (72.0–138.0)
LDL-cholesterol, mg/dl	84.0±23.0	73.9±26.6	89.7±21.9	73.4±18.1†
eGFR, ml·min^{-1}·1.73 m^{-2}	68.3±18.5	67.3±18.5	66.2±21.4	67.5±27.9
BNP, pg/ml	44.2 (19.7–72.1)	32.2 (17.8–62.0)	61.7 (23.8–100.5)	36.3 (16.9–99.0)
LVEF, %	62.2±7.7	62.1±6.8	63.7±7.7	64.8±6.5

Data are mean±SD, n (%) or median and (interquartile range).
*P<0.05 vs. control group at 6 months, †P<0.05 vs. baseline by the same treatment.
BMI, body mass index; BNP, B-type natriuretic peptide; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-insulin resistance; HOMA-β, HOMA β cell function; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

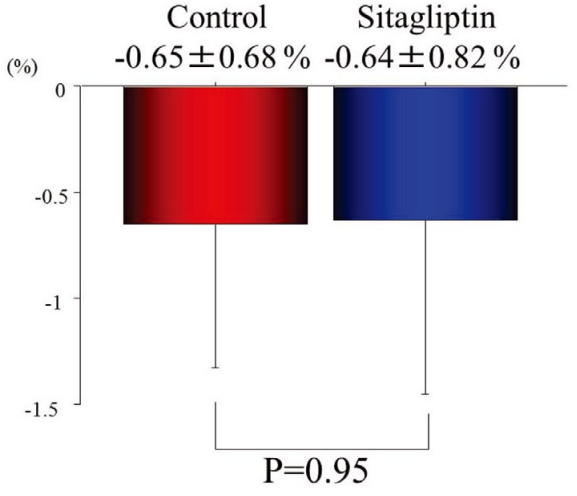

Figure 1. Change in hemoglobin A$_{1c}$ (HbA$_{1c}$) in patients who either received additional sitagliptin or underwent intensification of conventional treatment (control). The levels of HbA$_{1c}$ decreased to the same degree of magnitude in both groups (sitagliptin group: –0.64±0.82%, control groups: –0.65±0.68%, P=0.95).

the control group patient, the percent change in RHI was 15.2% (almost the average value). In the sitagliptin group, the 2 patients' percent changes in RHI were 44.4% and 26.7% (below the average value). Two patients were treated with ezemitibe during the follow-up period: 1 patient in the control group and 1 in the sitagliptin group. The control group patient's percent change in RHI was 15.3% (almost the average value), whereas the percent change in RHI for the patient in the sitagliptin group was 26.7% (below the average value). Only 1 patient in the sitagliptin group was treated with omega-3 polyunsaturated fatty acid during the follow-up period and this patient's percent change in RHI was 44.4% (below the average value). Consequently, the percent changes in RHI were not significantly different among the patients with or without additional ACEIs or ARBs, statins, ezemitibe or omega-3 polyunsaturated fatty acid in the present study. Furthermore, the sulfonylurea dose was reduced and finally ceased in 5 patients and 7 continued with the same dose of sulfonylurea after adding sitagliptin. The percent changes in RHI were not significantly different between the patients ceasing or continuing with sulfonylurea (ceased 75.1±75.9%; continued 68.1±74.3%, P=0.88). The level of hsCRP decreased significantly in the sitagliptin group (1.60 [0.45–2.85] to 0.70 [0.35–1.25] mg/L, P<0.01; Figure 3A), but not in the control group (1.60 [0.45–2.80] to 1.30 [0.30–3.95] mg/L, P=0.49; Figure 3B).

Linear regression analysis demonstrated a significant negative correlation between changes in RHI and hsCRP (r=–0.326; Figure 4), but not between RHI and HbA$_{1c}$ (r=–0.233, P=0.15). Furthermore, multivariate logistic regression analysis identified the additional treatment of sitagliptin as a significant and independent determinant of improved endothelial function (ΔRHI; after 6 months RHI–baseline RHI >0.11: median value), after adjustment for significant factors identified by univariate analysis (odds ratio: 6.63, 95% confidence interval: 1.28–34.25; Table 4).

Discussion

The present study clearly demonstrated that sitagliptin significantly improved peripheral endothelial dysfunction in association with its anti-inflammatory effects in patients with CAD and uncontrolled DM. To our knowledge, this is the first study to evaluate the effects of a DPP4 inhibitor on endothelial dysfunction in such patients.

Accumulating evidence implicates the involvement of endothelial dysfunction in all stages of atherogenesis.[3] In the

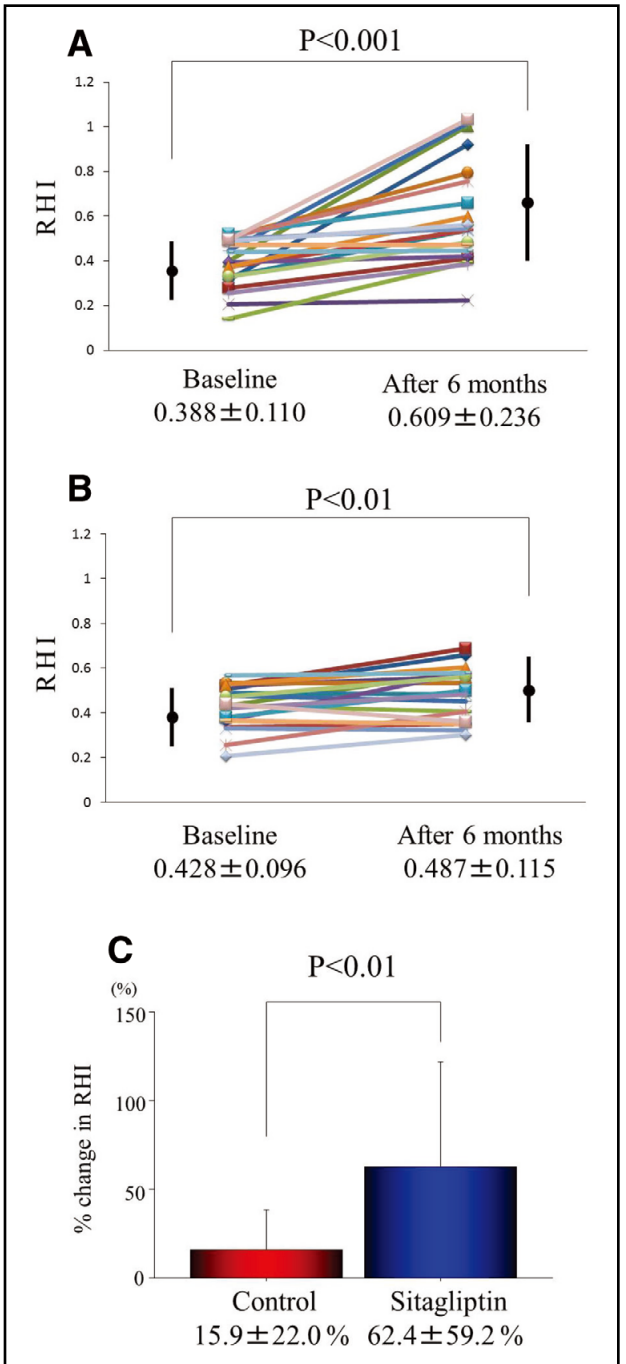

Figure 2. Change in the reactive hyperemia peripheral arterial tonometry index (RHI) in patients who either received additional sitagliptin or underwent intensification of conventional treatment (control). RHI values improved significantly by (**A**) sitagliptin treatment (0.388±0.110 to 0.609±0.236, P<0.001) and by (**B**) aggressive conventional treatment (0.428±0.096 to 0.487±0.115, P<0.01). (**C**) However, the percent RHI change in the sitagliptin group was significantly greater thant in the control group (62.4±59.2% vs. 15.9±22.0%, P<0.01).

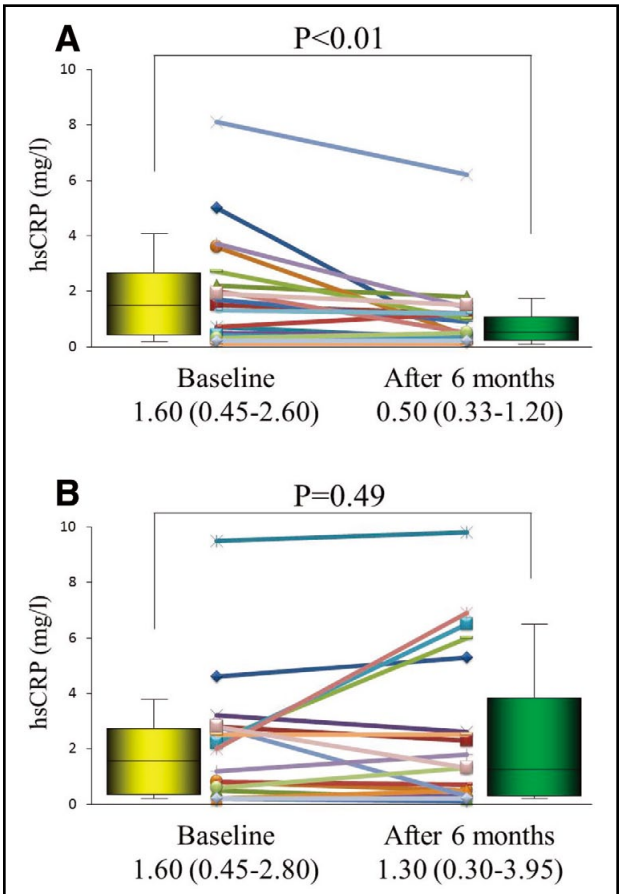

Figure 3. Change in high-sensitivity C-reactive protein (hsCRP) level in patients who either received additional sitagliptin or underwent intensification of conventional treatment (control). (**A**) Levels of hsCRP decreased significantly in the sitaglipitn group (1.60 [0.45–2.85] to 0.70 [0.35–1.25] mg/L, P<0.01). (**B**) For the control group, hsCRP levels at baseline and after 6 months were not different (1.60 [0.45–2.80] to 1.30 [0.30–3.95] mg/L, P=0.49).

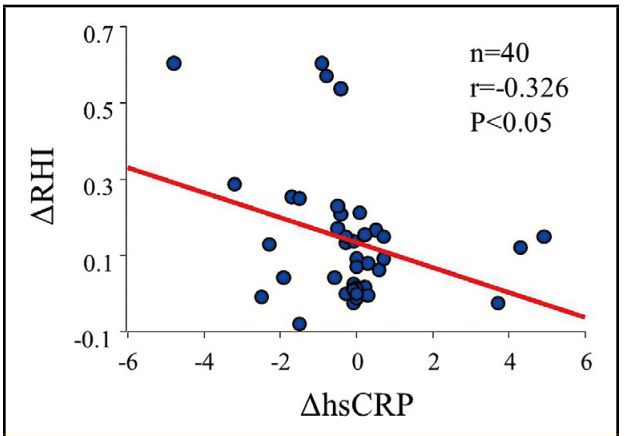

Figure 4. Correlation between the reactive hyperemia peripheral arterial tonometry index (RHI) and changes in high-sensitivity C-reactive protein (hsCRP) level. Linear regression analysis demonstrated a significant negative correlation between changes in RHI and hsCRP values (n=40, r=–0.326, P<0.05). ΔRHI; after 6 months RHI–baseline RHI; ΔhsCRP: after 6 months hsCRP–baseline hsCRP.

present study, although the levels of HbA_{1c} decreased similarly in both treatment groups, the RHI changes in the sitagliptin group were significantly greater than those in the control group, indicating that this DPP4 inhibitor improved peripheral endothelial dysfunction beyond its hypoglycemic

Table 4. Logistic Regression Analysis for Improvement in Endothelial Function

Factors	Univariate		Multivariate	
	OR (95% CI)	P value	OR (95% CI)	P value
Age, per year	1.01 (0.95–1.08)	0.73	–	
Sex, male	1.45 (0.43–5.20)	0.53	–	
ΔHbA$_{1c}$, per %	0.75 (0.32–1.82)	0.54	–	
ΔhsCRP, per g/dl	0.91 (0.62–1.31)	0.60	–	
ΔBMI, per kg/m^2	0.81 (0.49–1.33)	0.40	–	
ΔWC, per cm	0.83 (0.67–1.03)	0.10	–	
ΔSBP, per mmHg	0.96 (0.92–1.01)	0.07	–	
ΔDBP, per mmHg	1.01 (0.96–1.06)	0.72	–	
Δfasting glucose, per mg/dl	1.00 (0.98–1.02)	0.76	–	
Δfasting insulin, per μU/ml	1.25 (1.01–1.55)	<0.05	1.32 (0.99–1.73)	0.07
ΔHOMA-IR, per 1	1.45 (0.91–2.46)	0.11	–	
ΔHOMA-β, per 1	1.02 (0.99–1.04)	0.18	–	
ΔTC, per mg/dl	0.99 (0.97–1.01)	0.48	–	
ΔHDL-cholesterol, per mg/dl	0.97 (0.90–1.04)	0.36	–	
ΔTG, per mg/dl	1.00 (0.99–1.02)	0.74	–	
ΔLDL-cholesterol, per mg/dl	0.99 (0.96–1.01)	0.28	–	
ΔeGFR, per ml·min^{-1}·1.73 m^{-2}	1.00 (0.95–1.06)	0.90	–	
ΔBNP, per pg/ml	1.00 (0.98–1.02)	0.84	–	
ΔLVEF, per%	1.07 (0.96–1.20)	0.22	–	
Gensini score	1.01 (0.98–1.04)	0.20		
β-blockers, yes	2.43 (0.51–11.5)	0.26	–	
ACEIs or ARBs, yes	0.75 (0.17–3.33)	0.71	–	
CCBs, yes	1.26 (0.34–4.73)	0.74	–	
Nitrite, yes	1.59 (0.24–10.71)	0.63	–	
Insulin, yes	0.63 (0.10–4.24)	0.64	–	
Sulfonylurea, yes	1.50 (0.43–5.25)	0.53	–	
Biguanide, yes	1.00 (0.28–3.54)	0.99	–	
α-Glucosidase inhibitors, yes	0.54 (0.15–1.92)	0.34	–	
Pioglitazone, yes	1.00 (0.12–5.67)	0.99	–	
DPP4 inhibitor (sitagliptin), yes	5.44 (1.41–21.01)	<0.05	6.63 (1.28–34.25)	<0.05

DPP4, dipeptidyl peptidase-4; hsCRP, high-sensitivity C-reactive protein. Other abbreviations as in Tables 1,3.

action.

The extraglycemic effect of DPP4 inhibitors on endothelial function could reflect increased phosphorylation of endothelial nitric oxide synthase (eNOS). Nitric oxide, produced by eNOS, plays important roles in vascular homeostasis, including regulation and coordination of endothelial cell function, proliferation, senescence, and apoptosis,[17–19] as well as exhibiting atheroprotective effects.[20] Recently, we showed that des-fluoro-sitagliptin, a DPP4 inhibitor, improved high-fat diet-induced endothelial dysfunction by increasing the levels of phosphorylated eNOS in apolipoprotein-E-deficient mice, and thereby enhanced the phosphorylation of eNOS through the cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) pathway in human coronary artery endothelial cells, resulting in significantly decreased endothelial senescence and apoptosis.[10] Although we could not measure active GLP-1 levels in the present study, our previous studies suggested that sitagliptin could improve endothelial dysfunction by increasing eNOS activity through augmentation of the GLP-1 action on the endothelium. DPP4 can cleave active GLP-1 (7–36) into the less active form of GLP-1 (9–36) and there are these 2 forms of GLP-1 in the circulating plasma. It was previously reported that GLP-1 (7–36) could activate PKA-phosphoinositide 3-kinase/Akt-eNOS and cell proliferation, and that GLP-1 (9–36; cleaved form) could exert a similar effect to GLP-1 (7–36). Those authors suggested this was consistent with the existence of both GLP-1 receptor-dependent and -independent pathways in the regulation of endothelial function.[21] However, the concentration of GLP-1 (9–36; cleaved form) (ie, 100 nmol/L) in their study was much higher than physiological concentrations (ie, 100 pmol/L). Further examinations using the physiological concentration of GLP-1 (9–36; cleaved form) are needed.

Another extraglycemic effect of DPP4 inhibitors on endothelial function could be on other substrates of DPP4. Inhibition of DPP4 enzymatic activity could also modulate the activity of several proteins, such as stromal cell-derived factor-1α (SDF-1α) and neuropeptide Y.[22] It was reported previously that genetic and pharmacological inhibition of DPP4 combined with administration of granulocyte-colony stimulating factor improved survival after myocardial infarction through SDF-1α-dependent mobilization of cardiac stem cells in mice.[23] Furthermore, SDF-1α is upregulated and stimulates the bone marrow to release endothelial progenitor cells (EPCs) in response to ischemia.[24] EPCs provide vascular protection by means of endothelial repair and neoangiogenesis.[25] Sitagliptin also increases EPCs in type-2 DM patients, as an ancillary effect of DPP4 inhibition, possibly mediated through SDF-1α.[26]

These results suggest that sitagliptin improves endothelial dysfunction by increasing the number of EPCs through augmentation of SDF-1α. Although we did not measure SDF-1α and EPCs in the present study, we agree that this is an interesting hypothesis for future studies.

The third extraglycemic effect of DPP4 inhibitors on endothelial function could be an anti-inflammatory effect. We showed that a DPP4 inhibitor could reduce the mRNA expression levels of proinflammatory mediators (ie, interleukin (IL)-6, IL-1β, monocyte chemoattractant protein-1, and tumor necrosis factor-α) in the aorta of apolipoprotein-E-deficient mice. In vitro experiments also demonstrated that sitagliptin significantly increased GLP-1-induced cytosolic levels of cAMP compared with GLP-1 alone, resulted in inhibition of nuclear factor-κ B (NF-κB) p65 nuclear translocation through the cAMP/PKA pathway, and suppression of proinflammatory mediator production in response to lipopolysaccharide in human cultured macrophages. We further demonstrated in our in vitro experiments that sitagliptin significantly attenuated the expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in response to lipopolysaccharide and increased the phospolynation of eNOS through the cAMP/PKA pathway in human cultured coronary endothelial cells.[10] Furthermore, it was recently shown that treatment with sitagliptin in type-2 DM patients decreased the proinflammatory markers and inhibited NF-κB activation, resulted in falling plasma levels of CRP and IL-6.[27] In the present study, sitagliptin treatment for 6 months, but not intensification of conventional treatment, successfully decreased the level of hsCRP. This result suggests that sitagliptin improves endothelial dysfunction in part by improving the inflammatory state in cells of the arterial wall.

We evaluated peripheral endothelial function in our patients by RH-PAT, the results of which have been correlated with traditional and metabolic cardiovascular risk factors.[13,15,28] Bonetti et al demonstrated that the RH-PAT index also significantly predicted coronary endothelial dysfunction.[29] Furthermore, we previously showed that RH-PAT was predictive of CAD, especially non-obstructive CAD associated with coronary endothelial dysfunction, before angiography.[12] Because RH-PAT is a noninvasive, quantitative, and repeatable test, it could be an important index for evaluating vascular condition and treatment efficacy.

Study Limitations

First, this was not a blinded, randomized study. However, the characteristics of the patients were well matched between groups (Tables 1,3). Second, the sample size was small, and the design was a single-center study. We could not accurately investigate the effects of other drugs that might be expected to influence endothelial function such as ACEIs, ARBs, statins, ezemitibe, and omega-3 polyunsaturated fatty acids, because the number of patients in the subanalysis was small in the present study. Third, we did not assess the levels of GLP-1, glucose-dependent insulinotropic polypeptide (GIP) or SDF-1α, and other possible substrates of DPP4. Because we did not measure the concentrations of GLP-1 and GIP, it is unclear whether or not the improvement of endothelial dysfunction by sitagliptin depends on GLP-1. Furthermore, we could not measure the postprandial glucose levels, glycated-albumin levels, or oxidative stress markers in the present study. Rizzo et al have reported that DPP4 inhibitors reduced oxidative stress and inflammation in patients with DM.[30] A large multicenter double-blind study with assessment of postprandial glucose, glycated-albumin, oxidative stress markers, and the substrates of DPP4 is required to clarify the detailed mechanisms regarding our results. Further examinations are needed to determine the correlation between changes in endothelial function and change in the substrates of DPP4, including active GLP-1, GIP, and SDF-1α.

Conclusions

DPP4 inhibitor, sitagliptin, significantly improved endothelial dysfunction and inflammatory state, beyond its hypoglycemic action, in patients with CAD and uncontrolled DM, potentially providing beneficial effects on the cardiovascular system in such patients.

Acknowledgments

The authors are grateful to Megumi Nagahiro and Saeko Tokunaga from the Department of Cardiovascular Medicine, Faculty of Life Sciences, Graduate School of Medical Sciences, Kumamoto University, for skillful technical assistance.

Sources of Funding

This study was supported in part by a grant-in-aid for Scientific Research (C22590786 for S. Sugiyama) from the Ministry of Education, Science, and Culture in Japan, a grant support from Japan Heart Foundation Research Grant, Japan Heart Foundation/Novartis Grant for Research Award on Molecular and Cellular Cardiology, 2012, a Sakakibara Memorial Research Grant from the Japan Research Promotion Society for Cardiovascular Diseases, Banyu Life Science Foundation International, and Okukubo Memorial Fund for Medical Research in Kumamoto University School of Medicine.

Disclosures

None.

References

1. Saito I. Epidemiological evidence of type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in Japan. *Circ J* 2012; **76:** 1066–1073.
2. Buse JB, Ginsberg HN, Bakris GL, Clark NG, Costa F, Eckel R, et al. Primary prevention of cardiovascular diseases in people with diabetes mellitus: A scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2007; **115:** 114–126.
3. Vita JA, Keaney JF Jr. Endothelial function: A barometer for cardiovascular risk? *Circulation* 2002; **106:** 640–642.
4. Libby P. Inflammation in atherosclerosis. *Nature* 2002; **420:** 868–874.
5. Li AC, Glass CK. The macrophage foam cell as a target for therapeutic intervention. *Nat Med* 2002; **8:** 1235–1242.
6. Drucker DJ, Nauck MA. The incretin system: Glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368:** 1696–1705.
7. Kolterman OG, Buse JB, Fineman MS, Gaines E, Heintz S, Bicsak TA, et al. Synthetic exendin-4 (exenatide) significantly reduces postprandial and fasting plasma glucose in subjects with type 2 diabetes. *J Clin Endocrinol Metab* 2003; **88:** 3082–3089.
8. Nystrom T, Gutniak MK, Zhang Q, Zhang F, Holst JJ, Ahren B, et al. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 2004; **287:** E1209–E1215.
9. Yu M, Moreno C, Hoagland KM, Dahly A, Ditter K, Mistry M, et al. Antihypertensive effect of glucagon-like peptide 1 in Dahl salt-sensitive rats. *J Hypertens* 2003; **21:** 1125–1135.
10. Matsubara J, Sugiyama S, Sugamura K, Nakamura T, Fujiwara Y, Akiyama E, et al. A dipeptidyl peptidase-4 inhibitor, des-fluoro-sitagliptin, improves endothelial function and reduces atherosclerotic lesion formation in apolipoprotein e-deficient mice. *J Am Coll Cardiol* 2012; **59:** 265–276.
11. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; **51:** 606.
12. Matsuzawa Y, Sugiyama S, Sugamura K, Nozaki T, Ohba K, Konishi M, et al. Digital assessment of endothelial function and ischemic heart disease in women. *J Am Coll Cardiol* 2010; **55:** 1688–1696.

13. Hamburg NM, Keyes MJ, Larson MG, Vasan RS, Schnabel R, Pryde MM, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008; **117:** 2467–2474.
14. Selamet Tierney ES, Newburger JW, Gauvreau K, Geva J, Coogan E, Colan SD, et al. Endothelial pulse amplitude testing: Feasibility and reproducibility in adolescents. *J Pediatr* 2009; **154:** 901–905.
15. Bonetti PO, Barsness GW, Keelan PC, Schnell TI, Pumper GM, Kuvin JT, et al. Enhanced external counterpulsation improves endothelial function in patients with symptomatic coronary artery disease. *J Am Coll Cardiol* 2003; **41:** 1761–1768.
16. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53:** 982–992.
17. Ahanchi SS, Tsihlis ND, Kibbe MR. The role of nitric oxide in the pathophysiology of intimal hyperplasia. *J Vasc Surg* 2007; **45**(Suppl A)**:** A64–A73.
18. Moncada S, Palmer RM, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43:** 109–142.
19. Maron BA, Michel T. Subcellular localization of oxidants and redox modulation of endothelial nitric oxide synthase. *Circ J* 2012; **76:** 2497–2512.
20. Oemar BS, Tschudi MR, Godoy N, Brovkovich V, Malinski T, Luscher TF. Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. *Circulation* 1998; **97:** 2494–2498.
21. Erdogdu O, Nathanson D, Sjoholm A, Nystrom T, Zhang Q. Exendin-4 stimulates proliferation of human coronary artery endothelial cells through eNOS-, PKA- and PI3K/Akt-dependent pathways and requires GLP-1 receptor. *Mol Cell Endocrinol* 2010; **325:** 26–35.
22. Drucker DJ. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: Preclinical biology and mechanisms of action. *Diabetes Care* 2007; **30:** 1335–1343.
23. Zaruba MM, Theiss HD, Vallaster M, Mehl U, Brunner S, Fischer R, et al. Synergy between CD26/DPP-IV inhibition and G-CSF improves cardiac function after acute myocardial infarction. *Cell Stem Cell* 2009; **4:** 313–323.
24. Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004; **10:** 858–864.
25. Fadini GP, Agostini C, Sartore S, Avogaro A. Endothelial progenitor cells in the natural history of atherosclerosis. *Atherosclerosis* 2007; **194:** 46–54.
26. Fadini GP, Boscaro E, Albiero M, Menegazzo L, Frison V, de Kreutzenberg S, et al. The oral dipeptidyl peptidase-4 inhibitor sitagliptin increases circulating endothelial progenitor cells in patients with type 2 diabetes: Possible role of stromal-derived factor-1alpha. *Diabetes Care* 2010; **33:** 1607–1609.
27. Makdissi A, Ghanim H, Vora M, Green K, Abuaysheh S, Chaudhuri A, et al. Sitagliptin exerts an antinflammatory action. *J Clin Endocrinol Metab* 2012; **97:** 3333–3341.
28. Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003; **146:** 168–174.
29. Bonetti PO, Pumper GM, Higano ST, Holmes DR Jr, Kuvin JT, Lerman A. Noninvasive identification of patients with early coronary atherosclerosis by assessment of digital reactive hyperemia. *J Am Coll Cardiol* 2004; **44:** 2137–2141.
30. Rizzo MR, Barbieri M, Marfella R, Paolisso G. Reduction of oxidative stress and inflammation by blunting daily acute glucose fluctuations in patients with type 2 diabetes: Role of dipeptidyl peptidase-IV inhibition. *Diabetes Care* 2012; **35:** 2076–2082.