

Effects of a PPAR- γ (Peroxisome Proliferator-Activated Receptor-gamma) Activator on Flow-Mediated Brachial Artery Dilation and Circulating Level of microRNA-21 in Hypertensive Type 2 Diabetic Patients

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ABSTRACT

Background: Endothelial dysfunction has been documented in patients with type 2 diabetes especially when combined with hypertension. We prospectively investigated the effects of pioglitazone in improving endothelial function in hypertensive type 2 diabetic patients during the 6-month follow-up. **Methods:** Hypertensive type 2 diabetic patients were randomly assigned to pioglitazone (n = 25) or placebo (n = 25). Primary endpoint was to compare changes in brachial artery flow-mediated dilation (baFMD) between the 2 groups during the 6-month follow-up. Secondary endpoints were to compare changes in the circulating levels of microRNA-17, -21, 92a, -126, and -145 which have been known as indicators of endothelial cell migration and atherosclerosis progression during the 6-month follow-up. Inflammatory markers such as interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), high-sensitive C-reactive protein, adiponectin, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) were compared during the follow-up. **Results:** The prevalences of risk factors such as hyperlipidemia, smoking, stroke, and family history of coronary artery disease did not show significant differences between the 2 groups. Increases in baFMD (0.33 ± 0.34 mm vs. 0.02 ± 0.25 mm, $p < 0.05$, respectively) and in the level of circulating microRNA-21 (0.23 ± 0.05 vs. -0.06 ± 0.04 , $p < 0.05$, respectively) were significantly greater in the pioglitazone group when compared to the placebo group during the 6-month follow-up. No significant differences in the prevalences of new onset heart failure, fracture, and bladder cancer were noted during the follow-up between the 2 groups. Decreases in the levels of inflammatory marker such as IL-6 (-2.54 ± 2.32 pg/mL vs. -1.34 ± 2.12 pg/mL, $p < 0.05$, respectively), TNF- α (-1.54 ± 1.51 pg/mL vs. 0.14 ± 1.12 pg/mL, $p < 0.05$, respectively), sICAM-1 (-39 ± 52 ng/mL vs. 6 ± 72 ng/mL, $p < 0.05$, respectively), and sVCAM-1 (-154 ± 198 ng/mL vs. -11 ± 356 ng/mL, $p < 0.05$, respectively) were significantly greater in the pioglitazone group compared to the placebo group during the follow-up. **Conclusions:** In hypertensive type 2 diabetic patients, pioglitazone may increase baFMD and circulatory microRNA-21 and decrease inflammatory cytokines including IL-6, TNF- α , sICAM-1, and sVCAM-1.

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Introduction

Endothelial dysfunction is the first step in the progression of atherosclerosis.¹⁾ Endothelial dysfunction has

been more frequently documented in type 2 diabetic patients when combined with hypertension.^{1,2)} Hypertension is as an independent predictor for coronary heart disease, congestive heart failure, stroke, renal failure, and ischemic target vessel revascularization.^{3,4)} Hypertension often accompanies carbohydrate and lipid abnormalities as part of the metabolic syndrome,⁵⁾ and patients with the metabolic syndrome are at higher risk of cardiovascular morbidity and mortality.⁵⁾ Hypertension when combined with diabetes increase the risk of cardiovascular events.⁶⁻⁸⁾

Several studies highlight the beneficial effect of pioglitazone in reducing coronary atherosclerosis in type 2 diabetic patients.^{7,9)} However, the US Food and Drug Administration has informed the public that use of the pioglitazone for more than 1 year may be associated with an increased risk of bladder cancer especially for men.^{10,11)} A meta-analysis suggests that the pioglitazone confers excess risk for fractures especially for women.^{12,13)} Although anecdotal adverse events with pioglitazone have been reported, these findings have not been shown in large prospective, randomized studies. In our previous study, early decreases in the number of natural killer cells, circulating tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and monocyte chemoattractant protein-1 concentration, and the expression of chemokine receptor 2 on circulating CD14+ cells have been noted after pioglitazone treatment, subsequently reducing atherosclerosis progression in type 2 diabetic patients.⁷⁾ The early decreases in smooth muscle cell migration and proliferation in the pioglitazone group have been observed in type 2 diabetic patients. MicroRNAs are post-transcriptional regulators that bind to complementary sequences on target mRNAs.¹⁴⁾ The human genome may encode > 1,000 microRNAs and target about 60% of mammalian genes.¹⁴⁾ Aberrant expression of microRNAs implicated in numerous disease states. We prospectively investigated

the effects of pioglitazone in improving endothelial function, the changes in microRNA-17, -21, -92a, -126, -145, and the correlation between the changes in brachial artery flow-mediated dilation (baFMD) and microRNAs in hypertensive type 2 diabetic patients during the 6-month follow-up.

Subjects and methods

1. Study patients

Patients were eligible for this study if they were 45 to 75 years of age and had both essential hypertension and type 2 diabetes. Both previously treated and untreated hypertensive type 2 diabetic patients were prospectively included in the study at Korea University Anam Hospital cardiovascular centers from July 2011 through June 2012. A total of 85 patients were screened for inclusion in the study. Patients (n = 35) who did not fulfill the inclusion criteria or who had any of the exclusion criteria were excluded. Eligible patients (n = 50, 20 women and 30 men) were randomly assigned to receive either pioglitazone 15 mg (25 patients) or control (25 patients) after measuring baFMD. To be included in the study, a sitting diastolic blood pressure (SiDBP) of ≥ 80 mm Hg or a sitting systolic blood pressure (SiSBP) of ≥ 130 mm Hg had to be documented for previously untreated hypertensive type 2 diabetic patients. We excluded patients with the following conditions; the use of pioglitazone within 3 months before the enrollment, severe hypertension (SiSBP > 180 mm Hg and SiDBP > 110 mm Hg), acute coronary syndrome, and uncontrolled arrhythmia within three months. Patients with heart failure (ejection fraction < 45% or signs of heart failure), hepatic dysfunction (serum aspartate or alanine aminotransferase levels being above twice the upper limit of normal ranges), and serum creatinine > 2.0 mg/dL were

also excluded. Pregnant women, breastfeeding women, and women of childbearing potential who were not using appropriate contraceptive measures were also excluded in the study. Primary endpoint was to compare changes in baFMD between the 2 groups during the 6-month follow-up. Secondary endpoints were to compare changes in the circulating levels of microRNA-17, -21, 92a, -126, and -145 which have been known as indicators of endothelial cell migration and atherosclerosis progression during the 6-month follow-up. Inflammatory markers such as high-sensitive C-reactive protein (hsCRP), interleukin-6 (IL-6), TNF- α , adiponectin, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) were compared during the follow-up. The study was approved by the institute review board of Korea University Anam Hospital, and all participants gave written informed consent. The results were analyzed by the 'intention to treat' principle, and the initial administration of pioglitazone was used for analysis. Body mass index was calculated by dividing the square of the height in meters from the weight in kilograms.

2. Blood pressure measurements

Blood pressure was measured using mercury sphygmomanometer (WA Baum Co., Inc., Copiague, NY, USA) after 10 minutes of resting in a quiet room in the sitting position, and any medications or caffeine-containing drinks which may influence the blood pressure were not taken within 12 hours prior to the blood pressure measurement. A large cuff was used if the circumference of the arm was > 32 cm. SiDBP, SiSBP, and pulse rate were measured by a single investigator. Phase I and V Korotkoff sounds from the brachial artery were used as a systolic and a diastolic pressure, respectively. Heart rate was measured for one minute before blood pressure was measured.

3. Measurements of brachial artery flow-mediated dilation

Patients fasted for at least 8 h before the flow-mediated dilation (FMD) study; moreover, patients were educated not to exercise and not to ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least 4 hour before the study. Echocardiography (Vivid q; GE Healthcare, Milwaukee, WI, USA) was used for acquiring images, and a linear array transducer (12L, GE Healthcare) was used to acquire images with sufficient resolution. Patients were positioned supine with the arm in a comfortable position for imaging the brachial artery. The brachial artery was imaged above the antecubital fossa in the longitudinal plane. A segment with clear anterior and posterior intimal interfaces between the lumen and vessel wall was selected for continuous two-dimensional grayscale imaging. During image acquisition, anatomic landmarks such as veins and fascial planes were noted to help maintain the same image of the artery during the 6-month follow-up. To create a flow stimulus in the brachial artery, a blood pressure cuff was first placed above the forearm. Baseline rest images were acquired, and blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a midartery sample volume. Thereafter, arterial occlusion was created by cuff inflation to 180 mm Hg above systolic pressure to occlude arterial inflow for 5 minutes. Subsequent cuff deflation induced a brief high-flow state through the brachial artery, resulting in increase in shear stress with the brachial artery dilation. The longitudinal image of the artery was recorded continuously from 30 seconds before to 2 minutes after cuff deflation to measure percent change in diameter. A midartery pulsed Doppler signal was obtained upon immediate cuff release and 10 seconds after cuff deflation to assess hyperemic velocity.

4. Measurements of microRNA-17, -21, -92a, -126, -145

Peripheral blood samples (5 mL) were drawn into serum collection tubes, were allowed to stand for about 30 minutes at reverse transcription (RT) and were centrifuged at 1,800 g for 10 minutes at RT. The serum was aliquoted into eppendorf tubes and stored at -80°C. Total RNAs from human serum were isolated by using TRI Reagent BD (MRC, TB126) following the instructions from the manufacturer with modification. In brief, 250 µL of serum per eppendorf tube was added to 0.75 mL of TRI Reagent BD, was stored for 5 minutes at RT. The samples were extracted with 200 µL of chloroform, and the aqueous phase containing RNA was transferred to a fresh tube. The RNA was precipitated from the aqueous phase by centrifugation at 12,000 g 15 minutes 4°C after mixing with 500 µL of isopropanol. The RNA pellet was washed in 1 mL of 75% ethanol by centrifugation, and finally the pellet was re-suspended in 5 µL of RNase-free water. The samples isolated from the same patients were gathered. Total RNA was quantitated by using a spectrophotometer (ND-1000; NanoDrop Technologies, Wilmington, DE, USA). Ten ng of total RNA isolated from the serum was reverse transcribed in a 15 µL reaction volume using the TaqMan microRNA Reverse Transcription kit (Applied Biosystems, Grand Island, NY, USA) according to the instructions of the manufacturer. MiRNA-specific stem-loop RT primers, miR-17, miR-21, miR-92a, miR-126, miR-145, and miR-16 primers were used for RT reaction. Subsequently, 2 µL of the RT product was used for detecting miRNA expression by quantitative (q) polymerase chain reaction (PCR) using TaqMan microRNA Assay kits (Applied Biosystems) for the corresponding microRNA. Real-time PCR was performed using an iQ Cyclor (Bio-Rad Laboratories, Hercules, CA, USA) using the following program: 10 mi-

nutes pre-incubation at 95°C and 40 cycles of 15 seconds of denaturation at 95°C and 60 seconds of annealing/extending at 60°C. MiR-17, miR-21, miR-92a, miR-126, and miR-145 primers and miR-16 primers as an endogenous control were used. The amount of miRNA not detected after 40 cycles of a real-time PCR was regarded in the present study as a CT equivalent to 40. Negative controls were included with every real-time RT-PCR assay, and no amplification of the signal was observed when water was added instead of RNA or cDNA sample. The measurement of miRNA expression was assayed in duplicate. The Ct values were normalized to miR-16 and are expressed as $2^{-\{CT(\text{microRNA})-CT(\text{miR-16})\}}$.

5. Measurement of inflammatory markers

Inflammatory markers such as hsCRP, IL-6, TNF- α , adiponectin, sICAM-1, and sVCAM-1 were measured in both groups at the beginning of the study and at 6-month follow-up. Venous blood samples were drawn from each patient after eight hours or overnight fasting. Blood samples were centrifuged to obtain plasma, and the plasma was stored at -80°C. TNF- α was measured by a sandwich enzyme linked immuno sorbent assay (ELISA) with a minimum detectable level of 0.5 pg/mL (ALPCO Diagnostics, Salem, NH, USA). Undetectable TNF- α values were recorded as 0.4 pg/mL for one patient. IL-6 was also measured by a sandwich ELISA with a minimum detectable level of 0.16 pg/mL (ALPCO Diagnostics). The hsCRP concentrations were quantified using a latex nephelometer II (Dade Behring Inc., Newark, DE, USA). Plasma adiponectin concentration was assessed by radioimmunoassay (Linco Research Inc., St. Charles, MO, USA). The sensitivity of this assay was 0.78 ng/mL. The coefficients of variation for intra- and inter-assay were 9.3% and 15.3%, respectively. In addition, sICAM-1 and sVCAM-1 were measured using a commercially available

ELISA according to the manufacturer's instructions (R & D Systems, Minneapolis, MN, USA).

Inc., Chicago, IL, USA) was used for analyses.

Results

6. Statistical analysis

Data are expressed as mean \pm standard deviation for continuous variables, and data for the categorical variables are expressed as the number and the percentage of patients. Fisher's exact test or a chi-square test was used for categoric variables. The change from baseline was calculated as the value obtained at the end of treatment subtracted from the value obtained at the beginning of the intervention. The results between two groups were compared by an unpaired Student t-test, and the comparisons between before and after treatment were analyzed by a paired t-test. All analyses were performed according to the intention-to-treat principle. A p-value of less than 0.05 was considered significant. SPSS ver. 10.0 (SPSS

1. Study patients

Baseline patient characteristics of the pioglitazone group (n = 25) and the control group (n = 25) were not different (Table 1). The mean ages were not different (61.4 ± 12.4 years and 59.5 ± 11.8 years) as were the mean body mass index (24.8 ± 4.1 kg/m² and 24.1 ± 3.0 kg/m²) between the pioglitazone and control groups. The prevalences of hyperlipidemia, current smoking, and family history of coronary artery disease at baseline were not different in both groups. The number of patients taking ≥ 2 hypertensive medication (48.0% [n = 12] in the pioglitazone group vs. 52.0% [n = 13] in the control group, p = 0.777) were not different between the 2 groups. The

Table 1. Baseline patient characteristics

Variable	Pioglitazone group (n = 25)	Control group (n = 25)	p-value
Age (yr)	61.4 \pm 12.4	59.5 \pm 11.8	0.345
Male sex	16 (64.0)	14 (56.0)	0.564
Body mass index (kg/m ²)	24.8 \pm 4.1	24.1 \pm 3.0	0.394
Risk factors			
Hyperlipidemia	8 (32.0)	7 (28.0)	0.758
Current smoking	8 (32.0)	10 (40.0)	0.556
Family history of CAD	2 (8.0)	3 (12.0)	1.000
Past history of TIA or stroke	1 (4.0)	2 (8.0)	1.000
Insulin use	4 (16.0)	5 (20.0)	1.000
No. of hypertensive medication (≥ 2)	12 (48.0)	13 (52.0)	0.777
Hypertension medication			
ACE inhibitor	4 (16.0)	3 (12.0)	1.000
Angiotensin receptor blocker	8 (32.0)	9 (36.0)	0.765
Beta blocker	3 (12.0)	2 (8.0)	1.000
Calcium channel blocker	6 (24.0)	8 (32.0)	0.529
Diuretics	6 (24.0)	5 (20.0)	0.733
LVEF (%)	61.1 \pm 8.6	62.1 \pm 9.1	0.881

Values are presented as mean \pm standard deviation or number (%). The body mass index is the weight in kilograms divided by the square of the height in meters.

CAD, coronary artery disease; TIA, transient ischemic attack; ACE, angiotensin converting enzyme; LVEF, left ventricular ejection fraction.

baseline mean systolic blood pressure (SBP) medication (136 ± 13 in the pioglitazone group vs. 134 ± 12 in the control group, $p = 0.624$) and diastolic blood pressure (DBP) (79 ± 7 in the pioglitazone group vs. 76 ± 8 in the control group, $p = 0.482$) were not different between the two groups, and the prevalences of various active medications at baseline did not show significant differences. Dose of pioglitazone was doubled during the follow-up in 8 patients (32.0%) at physician's discretion.

2. Changes in flow-mediated dilatation and its correlation to microRNA-21

Increases in FMD during the 6-month follow-up were significantly greater in the pioglitazone group when compared to the control group (0.33 ± 0.34 mm vs. 0.02 ± 0.25 mm, $p < 0.05$, respectively) (Table 2, Fig. 1). Changes from baseline in the expression of microRNA-21 were significantly greater in the pioglitazone group when compared with the control group (0.23 ± 0.05 vs. $-0.06 \pm$

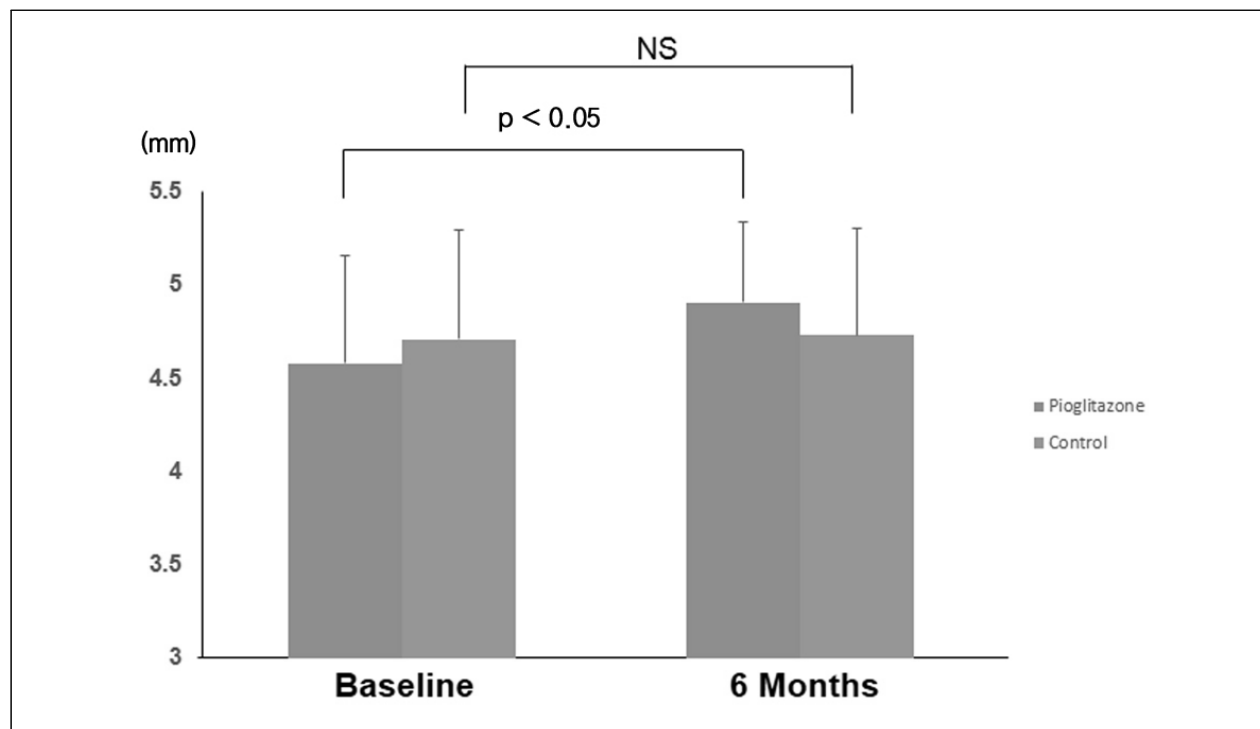


Fig. 1. Comparison of changes in brachial artery flow-mediated dilatation during the 6-month follow-up between the pioglitazone and control groups.

Table 2. Changes in brachial artery flow-mediated dilatation during the 6-month follow-up

Variable	Pioglitazone group (n = 25)		Control group (n = 25)	
	Baseline	After 6 months	Baseline	After 6 months
Brachial artery diameter at rest (mm)	4.47 ± 0.58	4.51 ± 0.49	4.59 ± 0.59	4.57 ± 0.57
Flow-mediated dilatation (mm)	4.58 ± 0.58	$4.91 \pm 0.43^{*,\dagger}$	4.71 ± 0.59	4.73 ± 0.58
Changes from at rest (mm)	0.11 ± 0.06	$0.40 \pm 0.08^{*,\dagger}$	0.12 ± 0.07	0.16 ± 0.07
Changes from baseline (mm)		$0.33 \pm 0.34^{\dagger}$		0.02 ± 0.25

* $p < 0.05$ compared with baseline. $^{\dagger}p < 0.05$ compared with the control group.

0.04, $p < 0.05$, respectively). Moreover, a strong positive correlation was noted between the changes in baFMD and the changes in the expression of microRNA-21 ($r = 0.72$, $p < 0.001$) (Fig. 2A, B). Changes in the expressions of microRNA-17, -92a, -126, -145) did not show significant differences between the 2 groups during the 6-month follow-up (Fig. 2C).

3. Changes in inflammatory markers and lipid profiles

Decreases in inflammatory markers such as IL-6 (-2.54 ± 2.32 pg/mL vs. -1.34 ± 2.12 pg/mL, $p < 0.05$, respectively), TNF- α (-1.54 ± 1.51 pg/mL vs. 0.14 ± 1.12 pg/mL, $p < 0.05$, respectively), sICAM-1 (-39 ± 52 ng/mL vs. 6 ± 72 ng/mL, $p < 0.05$, respectively), and sVCAM-1 (-154 ± 198 ng/mL vs. -11 ± 356 ng/mL, $p < 0.05$, respectively) were significantly greater in the pioglitazone group when compared to the control group during the 6-month follow-up (Table 3). Moreover, significant increases in adiponectin concentrations were found in the pioglitazone group when compared to the control group (1.47 ± 0.89 μ g/mL vs. 0.98 ± 0.88 μ g/mL, $p < 0.05$, respectively). However, no significant changes in hsCRP concentrations were noted between the 2 groups. Moreover, levels of total cholesterol and low density lipoprotein (LDL) cholesterol decreased significantly in both groups when compared to the baseline during the 6-month follow-up; however, changes from baseline showed no significant differences between the 2 groups (Table 4).

4. Clinical follow-up

The 6-month follow-up mean SBP medication (132 ± 12 in the pioglitazone group vs. 133 ± 11 in the control group, $p = 0.802$) and DBP (74 ± 6 in the pioglitazone group vs. 75 ± 7 in the control group, $p = 0.859$) were

not different between the two groups. No stroke occurred in both groups during the 6-month follow-up; moreover, the incidences of adverse clinical events such as death, myocardial infarction, new-onset heart failure, fracture, and bladder cancer were not different between the 2 groups (Table 5).

Discussion

Hypertensive type 2 diabetic patients are more prone to endothelial dysfunction, leading to atherosclerosis progression.¹⁵⁾ A diabetic medication which also improves endothelial dysfunction can be of significant clinical benefit to diabetic patients especially when combined with hypertension. Increasing interest remains in the identification of systemic pharmacological therapies to improve endothelial dysfunction and prevent atherosclerosis progression. The thiazolidinediones such as pioglitazone interact with the peroxisome proliferator-activated receptor (PPAR)- γ ligand-binding domain and have been known for their hypoglycemic effect, controlling dyslipidemia, and reducing inflammation.^{7,16)} The nuclear hormone receptor PPAR- γ is activated by its ligand, and PPAR- γ functions as a transcriptional regulator of multiple genes regulating glucose and lipid metabolism.^{7,16)} In addition to their hypoglycemic effect and correcting dyslipidemia, pioglitazone demonstrated antiatherogenic effects in vascular cells in vitro.⁹⁾ Pioglitazone, with its anti-inflammatory and antiproliferative properties, could be an appropriate therapeutic option for treating endothelial dysfunction in hypertensive patients with type 2 diabetes.^{7,17)} The purpose of this prospective, randomized, single-blinded, investigator-initiated, 6-month follow-up study was to compare the effects of pioglitazone on brachial artery endothelial function, inflammatory markers, and circulating microRNAs in hypertensive type 2 diabetic patients.

This is the first prospective, randomized study to dem-

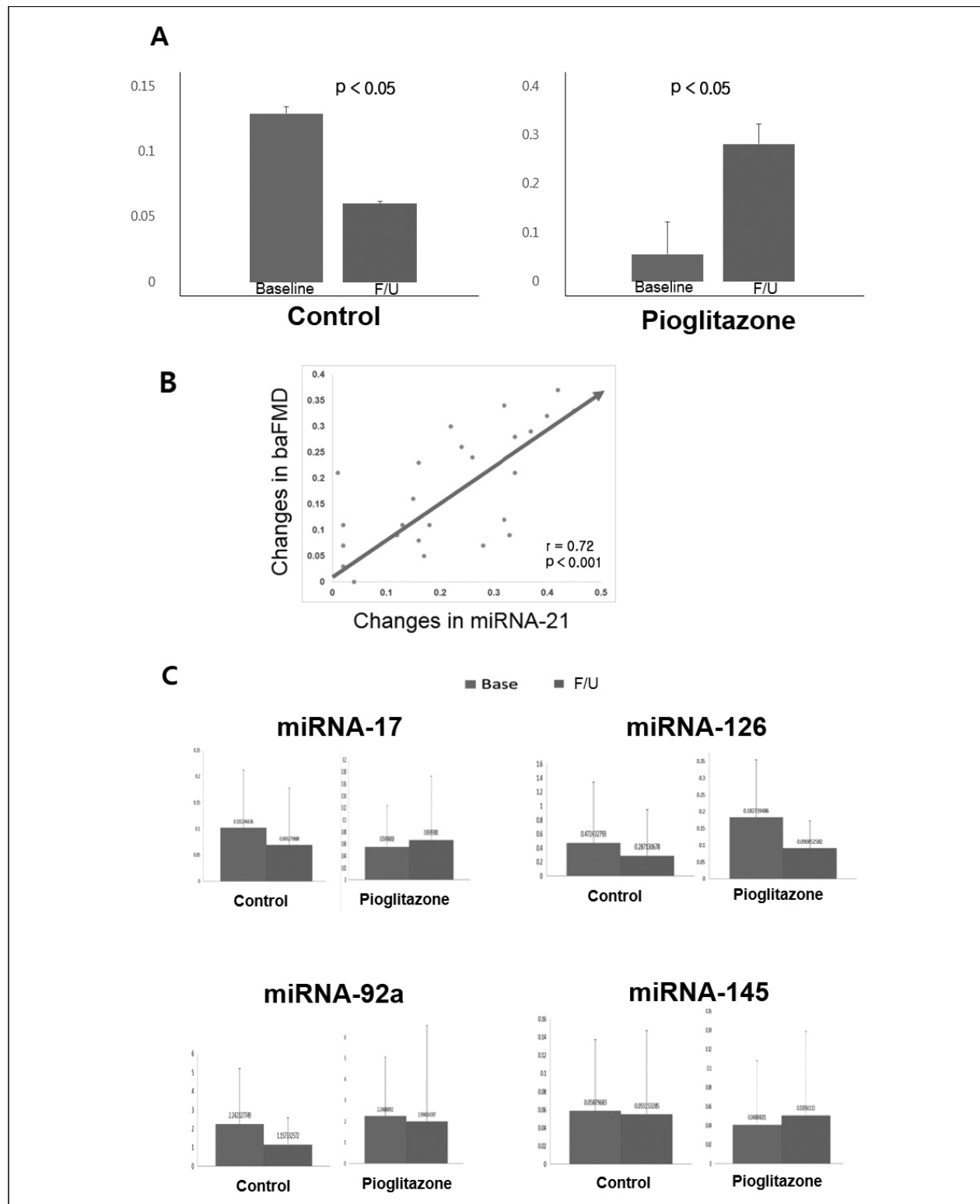


Fig. 2. (A) MicroRNA-21 increased significantly only in the pioglitazone group during the 6-month F/U, and (B) significant correlation was found between the changes in microRNA-21 and changes in brachial artery flow-mediated dilatation. (C) No significant differences were found with changes in microRNA-17, -92a, -126, and -145. baFMD, brachial artery flow-mediated dilatation; F/U, follow-up.

Table 3. Changes in the level of inflammatory markers during the 6-month follow-up

Variable	Pioglitazone group (n = 25)		Control group (n = 25)	
	Baseline	After 6 months	Baseline	After 6 months
Interleukin-6 (pg/mL)	3.91 ± 3.12	1.45 ± 1.10*	3.56 ± 3.21	2.23 ± 1.71*
Changes from baseline (pg/mL)		-2.54 ± 2.32 [†]		-1.34 ± 2.12
Tumor necrosis factor- α (pg/mL)	5.71 ± 4.19	4.16 ± 2.56*	5.16 ± 3.41	4.99 ± 3.11
Changes from baseline (pg/mL)		-1.54 ± 1.51 [†]		0.14 ± 1.12
High-sensitive C-reactive protein (mg/L)	4.89 ± 4.11	1.80 ± 1.57*	5.11 ± 4.22	1.89 ± 1.81*
Changes from baseline (mg/L)		-3.09 ± 3.01		-3.21 ± 2.98
Adiponectin (μ g/mL)	5.29 ± 4.17	6.76 ± 4.14*	5.55 ± 3.85	6.49 ± 4.66*
Changes from baseline (μ g/mL)		1.47 ± 0.89 [†]		0.98 ± 0.88
sICAM-1 (ng/mL)	430 ± 154	391 ± 133*	416 ± 131	422 ± 172
Changes from baseline (ng/mL)		-39 ± 52 [†]		6 ± 72
sVCAM-1 (ng/mL)	1107 ± 344	954 ± 328*	1088 ± 429	1077 ± 401
Changes from baseline (ng/mL)		-154 ± 198 [†]		-11 ± 356

Values are presented as mean ± standard deviation.

sICAM-1, soluble intercellular adhesion molecule-1; sVCAM-1, soluble vascular cell adhesion molecule-1.

*p < 0.05 compared with baseline. [†]p < 0.05 compared with the control group.

Table 4. Changes in the level of lipid profiles during the 6-month follow-up

Variable	Pioglitazone group (n = 25)		Control group (n = 25)	
	Baseline	After 6 months	Baseline	After 6 months
Total cholesterol (mg/dL)	220 ± 54	144 ± 41*	215 ± 44	141 ± 32*
Changes from baseline (mg/dL)		-76 ± 62		-75 ± 43
Low density lipoprotein cholesterol (mg/dL)	143 ± 73	73 ± 53*	146 ± 108	77 ± 39*
Changes from baseline (mg/dL)		-70 ± 39		-68 ± 42
High density lipoprotein cholesterol (mg/dL)	44 ± 27	46 ± 18	40 ± 22	45 ± 12
Changes from baseline (mg/dL)		2 ± 9		4 ± 7
Triglyceride (mg/dL)	127 ± 71	110 ± 94	123 ± 74	113 ± 69
Changes from baseline (mg/dL)		-16 ± 70		-10 ± 59

Values are presented as or mean ± standard deviation.

*p < 0.05 compared with baseline. [†]p < 0.05 compared with the control group.

Table 5. Comparison of adverse clinical events between the 2 groups during the 6-month follow-up

Variable	Pioglitazone group (n = 25)	Control group (n = 25)	p-value
Death	0 (0.0)	0 (0.0)	NA
Myocardial infarction	0 (0.0)	1 (4.0)	1.000
New onset CHF	1 (4.0)	0 (0.0)	1.000
Fracture	1 (4.0)	0 (0.0)	1.000
Stroke	0 (0.0)	0 (0.0)	NA
Bladder cancer	0 (0.0)	0 (0.0)	NA

Values are presented as number (%).

CHF, congestive heart failure; NA, not available.

onstrate the effect of pioglitazone in improving endothelial dysfunction and in increasing microRNA-21 during the 6-month follow-up. To the best of my knowledge, this is the first study demonstrating the association between the use of pioglitazone and the increases in microRNA-21, and a significant positive correlation between the changes in baFMD and changes in microRNA-21 was noted during the 6-month follow-up ($r = 0.72$, $p < 0.001$). MicroRNAs are a class of endogenous, small, non-coding RNAs that regulate expression of the protein coding genes via degradation or translational inhibition of their target messenger RNAs.¹⁸⁾ After screening endothelial function-related microRNAs such as microRNA-17, -21, -92a, -126, -145, -24, -26, -143, -155, -423, -1, -10a, -100, -204, and -208a in peripheral blood of patients with ($n = 3$) or without ($n = 5$) endothelial dysfunction in our pilot study, high expression of microRNA-17, -21, -92a, -126, and -145 were noted. MicroRNA-21 has been known for regulating target proteins which are involved in cellular survival, apoptosis and cell invasiveness.^{18,19)} MicroRNA-21 is known to play critical parts in the pathogenesis of cardiac cell growth and death, smooth muscle cell proliferation and apoptosis, and cardiac fibroblast activation.^{19,20)} Expression of microRNA-21 is deregulated in arteries and cardiomyocytes in patients with cardiovascular diseases such as cardiac hypertrophy, congestive heart failure, and ischemic heart disease. Furthermore, endothelial dysfunction, which is an indication of early atherosclerosis progression, could be identified with increasing changes in circulating microRNA-21 in hypertensive type 2 diabetic patients in this study. Although other microRNAs such as microRNA-17, -92a, -126, and -145 demonstrated as potential indicators of endothelial function in other studies,^{18,20-23)} same trends were not demonstrated in our study confined to the hypertensive type 2 diabetic patients during the 6-month follow-up.

There are several possible mechanisms to the significant improvement in endothelial dysfunction in the pioglitazone group when compared to the control group during the follow-up, even though the extents of blood pressure reduction were similar with either treatment during the follow-up. The inflammatory markers such as IL-6, TNF- α , sICAM-1, and sVCAM-1 decreased significantly in the pioglitazone group when compared with the control group, thereby reducing inflammatory condition during the follow-up. The anti-inflammatory and antiproliferative properties of adiponectin depend on its suppressing effect on adipose tissue and macrophages, thereby reducing the production and activity of pro-inflammatory cytokines such as IL-6 and TNF- α .²⁴⁻²⁶⁾ The significant increase in adiponectin concentration only in the pioglitazone group may have caused the decreased production of IL-6 and TNF- α from both adipose tissue and macrophages, subsequently improving endothelial dysfunction. The increase in adiponectin concentrations with pioglitazone was associated with the improvement in endothelial function in the present study, and this finding suggests that adiponectin may be involved in the early processes of atherosclerosis. Adiponectin, which has been known for decreasing inflammatory cytokines and for interfering with TNF- α signaling, increased significantly only in the pioglitazone group.²⁶⁾ Expression of adhesion molecules such as sICAM-1 and sVCAM-1 decreased significantly only in the pioglitazone group, correlating with the improvement in brachial artery endothelial function in this study. By improving endothelial function, expression of early atherosclerosis markers such as sICAM-1 and sVCAM-1 could be suppressed, thereby decreasing circulating monocyte adhesion and accumulation in the arterial intima.²⁷⁾

Total cholesterol and LDL cholesterol concentrations significantly decreased compared with baseline in both

groups at 6-month follow-up since atorvastatin was administered in more than 75% of patients in both groups (76% [n = 19] in the pioglitazone group vs. 88% [n = 22] in the control group, $p = 0.463$). Although no significant differences were noted in lipid profiles during the follow-up, co-administration of pioglitazone with atorvastatin provided additional effects on endothelial function and circulating inflammatory markers.⁷⁾ Additive pleiotropic effect of pioglitazone on lipid metabolism in combination with atorvastatins may have caused greater improvement in brachial artery endothelial function in hypertensive type 2 diabetic patients in this study, and this treatment combination of pioglitazone and atorvastatin may benefit hypertensive type 2 diabetic patients with endothelial dysfunction even during the short-term follow-up. There is a few limitation to the present study. Multicenter studies with large number of hypertensive type 2 diabetic patients are needed to confirm the beneficial effects of pioglitazone in improving endothelial dysfunction. Moreover, the exact molecular mechanisms underlying the anti-inflammatory and antiatherogenic activities of pioglitazone still remains to be verified, and the number of study population was not enough to draw any conclusions about adverse clinical events such as new-onset heart failure, fracture, stroke, and bladder cancer during the relatively short period of follow-up.

Pioglitazone not only adjusted glucose and lipid metabolism but also regulated inflammation and circulating microRNA-21 levels, thereby eventually improving endothelial dysfunction in hypertensive type 2 diabetic patients. Therefore, hypertensive type 2 diabetic patients treated with pioglitazone not only benefit from its hypoglycemic effects but also from its anti-inflammatory and anti-atherogenic effects. In conclusion, PPAR- γ activation by pioglitazone may provide new therapeutic options in the management of hypertensive type 2 diabetic patients with

endothelial dysfunction, and circulating microRNA-21 could be used as an indicator of improvement in endothelial function in hypertensive type 2 diabetic patients.

Summary

연구배경: 고혈압을 동반한 제2형 당뇨병 환자에게 PPAR- γ 활성화제인 pioglitazone을 6개월 사용하였을 때 상완동맥내피세포 기능에 어떠한 영향을 주며 혈관내피세포 기능에 관여하는 혈중 microRNA-17, -21, -92a, -126, -145에 어떠한 영향을 주는지 알아보하고자 하였다.

방법: 고혈압을 동반한 제2형 당뇨병 환자에게 pioglitazone군(25명)과 대조군(25명)으로 무작위 배정 후 상완동맥 혈류 관련성 확장검사, 혈중 microRNA-17, -21, -92a, -126, -145의 변화와 혈중 염증성 지표인 interleukin-6 (IL-6), tumor necrosis factor- α (TNF- α), high-sensitive C-reactive protein, adiponectin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1)을 내원 시와 6개월 후 반복 측정하였다.

결과: 양 군 간에 위험인자의 비율은 통계적 차이가 없었다. Pioglitazone군에서 시술 전과 6개월에 시행한 상완동맥 혈류 관련성 확장검사에서 유의하게 증가하였으며 (0.33 ± 0.34 mm vs. 0.02 ± 0.25 mm, $p < 0.05$, respectively), 혈중 microRNA-21도 유의하게 증가하였다 (0.23 ± 0.05 vs. -0.06 ± 0.04 , $p < 0.05$, respectively). 혈중 염증 마커인 IL-6 (-2.54 ± 2.32 pg/mL vs. -1.34 ± 2.12 pg/mL, $p < 0.05$, respectively), TNF- α (-1.54 ± 1.51 pg/mL vs. 0.14 ± 1.12 pg/mL, $p < 0.05$, respectively), ICAM-1 (-39 ± 52 ng/mL vs. 6 ± 72 ng/mL, $p < 0.05$, respectively), and VCAM-1 (-154 ± 198 ng/mL vs. -11 ± 356 ng/mL, $p < 0.05$, respectively)도 pioglitazone군에서 유의하게 감소하였다. 새롭게 발생한 심부전, 골절, 방광암의 발생에 있어서는 양 군 간 뚜렷한 차이는 없었다.

결론: 고혈압을 동반한 제2형 당뇨병 환자에게 pioglitazone을 6개월 사용하였을 때 상완동맥내피세포 기능을

개선시켰으며, 혈중 microRNA-21의 의미 있는 증가를 확인할 수 있었다. 혈중 microRNA-21의 증가를 상완동맥내피세포 기능 개선의 지표로 활용할 수 있을 것이다.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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