Effect of Cilostazol Treatment on Adiponectin and Soluble CD40 Ligand Levels in Diabetic Patients With Peripheral Arterial Occlusion Disease

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Background: Peripheral arterial occlusion disease (PAOD) is caused mainly by chronic inflammation and endothelium dysfunction, and is often treated with cilostazol. However, because this drug's influence on atherogenic cytokines is still not well known, this study examined the effect of cilostazol on the serum levels of soluble CD40 ligand (sCD40L), adiponectin and high-sensitivity C-reactive protein (hs-CRP) in patients with type 2 diabetes and PAOD.

Methods and Results: The 92 type 2 diabetics with PAOD and 100 non-PAOD diabetics were enrolled and randomly assigned to a group receiving either cilostazol or placebo for 6 months. The atherogenic cytokines were measured at the beginning and completion of the study. In the PAOD groups, those in the cilostazol group had significant changes in the levels of hs-CRP, sCD40L and adiponectin ($P=0.001$, $P=0.05$, $P=0.004$, respectively). Changes in the levels of adiponectin and sCD40L were more significant in the PAOD group treated with cilostazol than in the non-PAOD group also treated with the drug ($P=0.01$ and $P=0.008$, respectively).

Conclusions: Cilostazol can decrease hs-CRP and sCD40L levels and increase that of adiponectin, and then delay the progression of atherogenesis and chronic inflammation in type 2 diabetics, especially those with PAOD. (Circ J 2009; 73: 948–954)

Key Words: Adiponectin; CD40; Cilostazol; Diabetes mellitus; Peripheral arterial disease

Peripheral arterial occlusion disease (PAOD) is a common manifestation of atherosclerosis in patients with type 2 diabetes mellitus (DM).^{1,2} In Taiwan, approximately 33–50% of patients undergoing lower extremity amputation have DM, and more than 50% of them have PAOD.^{3,4} The risk factors associated with PAOD are similar to those for coronary artery disease and cerebrovascular disease.

It has been proved that atherosclerosis-related macroangiopathy is a result of chronic inflammation and endothelium dysfunction. Levels of high-sensitivity C-reactive protein (hs-CRP) and adiponectin have been found to be markers of chronic inflammation in many human studies.^{5–7} Interaction of the multipotent immunomodulator CD40 ligand with its receptor is also as an important contributor to chronic inflammation and endothelium dysfunction. The soluble form of the CD40 ligand (sCD40L), a marker of proinflammatory and platelet activation, is high in the sera of DM patients with coronary artery disease.⁸ However, few studies have investigated the relationships between PAOD and adiponectin and sCD40L levels.

Cilostazol, a vasodilator and inhibitor of platelet aggregation, has been used in the treatment of chronic PAOD since 1988. Because little is known regarding its effects on PAOD and the aforementioned atherogenic cytokines in DM patients, we performed a randomized, placebo-controlled, single-blinded trial to examine the effect of cilostazol treatment on the serum levels of sCD40L, adiponectin and hs-CRP in patients with type 2 DM.

Methods

Subjects

We enrolled 92 PAOD patients (53 women, 39 men; mean age 63.3 ± 18.8 years) and 100 non-PAOD patients as controls (58 women, 42 men; mean age 63.0 ± 14.7 years) at our outpatient clinic from 2006 to 2008. Sex ratio and ranges of age and body mass index (BMI) were considered while selecting the controls. All patients were confirmed to have type 2 DM based on the 2005 American Diabetes Association diagnostic criteria and had received oral anti-diabetic drugs (OADs) for more than 6 months. Because some antiplatelet, antihypertension and antihyperlipidemia drugs have an antiinflammatory effect, the dosages of all medications, including OADs, aspirin, antihypertension drugs and antihyperlipidemia drugs, were not changed for 6 months prior to the study or during the study period. We excluded any patient who had received insulin injections for more than 1 month or had been found to have had cardiogenic shock, unstable angina, old stroke, or myocardial infarction during the preceding 6 months, had an ankle systolic blood pressure (SBP) ≥ 200 mmHg, had been treated with insulin, and had impaired renal function (creatinine level >1.4 mg/dl), cancer, systemic inflammatory disease or

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Table 1. Characteristics of the Control and Patient Groups

	PAOD group	Non-PAOD group	P value
Age (years)	63.3±18.8	63.0±14.7	NS
Sex (M/F)	39/53	42/58	NS
BMI (kg/m ²)	26.0±4.5	25.9±3.9	NS
WC (cm)	98.7±11.9	98.4±10.0	NS
BF (%)	27.1±5.9	26.8±6.5	NS
SBP (mmHg)	124±22.5	127±23.8	NS
DBP (mmHg)	74±12.4	72±10.9	NS
TC (mg/dl)	188.3±35.8	185±50.9	NS
LDL (mg/dl)	119.9±29.4	115±33.7	NS
HDL (mg/dl)	49.4±13.5	50.1±14.0	NS
TG (mg/dl)	147.6±82.0	151±56.4	NS
HbA _{1c} (%)	8.6±2.1	8.2±1.1	0.06
HOMA IR	4.22±1.21	3.98±1.19	NS
hs-CRP (ng/ml)	7.2±4.5	2.5±1.5	<0.001
Adiponectin (μg/ml)	7.8±5.8	9.1±2.9	<0.001
sCD40L (ng/ml)	8.6±4.9	8.1±3.1	0.01
ABI (right leg)	0.76±0.11	1.15±0.13	<0.001
ABI (left leg)	0.69±0.18	1.16±0.09	<0.001
Comorbidity			
Hypertension (%)	36.5	38.0	NS
Hyperlipidemia (%)	40.6	38.0	NS
Drugs			
Antihypertensive	34.4	35.0	NS
Antiplatelet	65.6	63	NS
Lipid-lowering	40.6	38	NS

PAOD, peripheral arterial occlusion disease; BMI, body mass index; WC, waist circumference; BF, body fat; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglyceride; HbA_{1c}, hemoglobin A_{1c}; HOMA IR, homeostasis model assessment insulin resistance; hs-CRP, high-sensitive C-reactive protein; sCD40L, soluble CD40 ligand; ABI, ankle-brachial index.

had ever been treated with cilostazol. We also excluded anyone who had a history of smoking or had undergone leg surgery.

PAOD was defined as ankle-brachial index (ABI) <0.9 in either leg, as described in previous studies of PAOD patients with type 2 DM.^{3,4} The diabetic patients with PAOD and those without PAOD were randomly assigned to a 6-month treatment group receiving either cilostazol (100 mg twice daily) or placebo. The study was conducted according to the guidelines of the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of the Chang Gung Memorial Hospital. Written informed consent was given by all subjects prior to their participation.

After resting at least 30 min while supine on a bed, ABI measurements were performed in a room maintained at a temperature of 23–25°C. The ABI was calculated as the ratio of ankle SBP to the brachial SBP, both of which were measured by an automatic device (form PWV/ABI; AT, Komaki, Aichi, Japan).

The following clinical and laboratory parameters were measured at the beginning and completion of the study: BMI, blood pressure (BP) waist circumference (WC), % body fat (%BF), ABI and the serum levels of total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), hemoglobin A_{1c} (HbA_{1c}), fasting blood glucose (FBG), insulin, hs-CRP, adiponectin and sCD40L. The blood samples from each patient were obtained after an overnight fast that began at midnight. Patients were requested to wear light clothing during the measurements of weight, height, and BMI. The %BF was measured by electrical bioimpedance (BioDynamic Model 310 Body Composition Analyzer; Seattle, WA, USA) after a 4-h fast. The serum samples for hs-CRP, sCD40L and adiponectin levels were frozen at –70°C until used. All samples from the

same patient were run in the same assay and every sample was measured 3 times to obtain an average value.

Assays

The concentrations of FBG, TC, LDL, HDL and TG were measured using an autoanalyser (Hitachi 7250 Special; Hitachi, Tokyo, Japan). The HbA_{1c} level was measured by high-pressure liquid chromatography (Bio-Rad Laboratories, Richmond, CA, USA), and the plasma hs-CRP level was measured by a high-sensitivity monoclonal antibody assay (Dade Behring Marburg GmbH, Germany).

Plasma insulin concentrations were determined by automated immunoassay (Access; Beckman Instruments, Fullerton, CA, USA). Insulin resistance (IR) was determined by homeostasis model assessment (HOMA) and calculated using the FBG and fasting insulin levels in each participant:

$$\text{HOMA IR} = \frac{\text{fasting glucose (mmol/L)} \times \text{fasting insulin (mU/ml)}}{22.5}$$

The fasting serum adiponectin concentration was measured using a commercially available ELISA kit (Human Adiponectin ELISA kit, Linco Research, MO, USA). The level of circulating sCD40L was also determined by ELISA kit (Bender Med Systems, Vienna, Austria).

Statistical Analysis

Differences between groups were analyzed using a non-parametric Wilcoxon test. The relationships between variables were analyzed by Pearson correlation and stepwise multiple regression analyses. Differences between time points in treatment were calculated using repeated measures of general linear model. All data are reported as mean ± SD. A probability value <0.05 was regarded as significant. All

Figure. Correlation between ankle-brachial index (ABI) and chronic inflammation markers: high-sensitivity C-reactive protein (CRP), adiponectin and soluble CD40 ligand.

statistical operations were performed using the Statistical Package for Social Science program (SPSS for Windows, Version 11.5; SPSS, Chicago, IL, USA).

Results

PAOD and Non-PAOD Patients

Table 1 compares the different parameters between PAOD and non-PAOD patients. Although the 2 groups were similar in age, sex ratio, BP, BMI, WC, %BF and similar serum levels of TC, LDL, HDL, TG, HOMA IR and HbA_{1c}, the PAOD group had higher serum levels of hs-CRP and sCD40L and a lower adiponectin level and ABI than the non-PAOD group.

Correlation Between ABI and Other Parameters

In all patients, ABI negatively correlated with age and the levels of hs-CRP and sCD40L ($r = -0.79, P < 0.001$; $r = -0.39, P = 0.01$; $r = -0.40, P = 0.007$, respectively) and positively correlated with adiponectin level ($r = 0.311, P < 0.001$)

(**Figure**). There was no apparent correlation between ABI and the other parameters, including BMI, %BF, BP or WC or levels of TC, LDL, HDL, TG, HOMA IR or HbA_{1c}. We used linear regression analysis to predict the effect of each of the measured parameters on ABI in PAOD patients (**Table 2**). Our multivariate regression analysis, which was performed using generalized estimating equations, revealed that age and adiponectin levels were significantly strong predictors for ABI ($P < 0.0001$ and $P = 0.023$, respectively).

Changes in the Tested Parameters After Cilostazol Treatment

Table 3 presents the changes in the tested parameters in the PAOD group after 6 months of treatment with cilostazol, and in the PAOD group after 6 months of treatment with placebo. In the PAOD groups treated with cilostazol, there were no significant changes in ABI, BMI, WC, or %BF or the levels of TC, LDL, HDL, TG, HOMA IR or HbA_{1c} (tests of within-subjects contrasts, $P > 0.05$). However, there were significant changes in the levels of

Table 2. Multiple Regression Analysis of the Relationships Among Age, BMI, %BF, WC and the Levels of HbA_{1c}, TC, LDL, HDL, TG, hs-CRP, Adiponectin and sCD40L

	Regression coefficient	SE	Standardized regression coefficient	P value
Age	0.397	0.34	-0.38	<0.0001
Adiponectin	0.157	0.18	0.11	0.023

Dependent variable: ABI. R² for the entire model=0.54.

Independent variables: age, BMI, %BF, WC HOMA IR and levels of HbA_{1c}, TC, LDL, HDL, TG, hs-CRP, adiponectin and sCD40L.

See Table 1 for abbreviations.

Table 3. Effect of Cilostazol in PAOD Patients

	Cilostazol group		Placebo group		P value	P* value
	Before treatment	After treatment	Before treatment	After treatment		
Age (years)	62.6±9.5		63.8±9.4			NS
Sex (M/F)	19/22		20/21			NS
BMI (kg/m ²)	26.2±5.5	26.1±3.3	25.8±4.1	26.3±4.4	NS	NS
WC (cm)	98.2±12.2	98.2±9.6	99.2±9.9	97.2±9.8	NS	NS
BF (%)	27.3±5.9	27.8±6.5	27.0±5.3	27.3±7.2	NS	NS
TC (mg/dl)	190.1±29.4	188.8±35.8	186.5±35.8	189.9±41.0	NS	0.02
LDL (mg/dl)	119.8±30.4	108.7±33.8	120.0±28.4	119.0±32.9	NS	0.04
HDL (mg/dl)	48.3±12.1	51.3±11.6	50.5±13.0	49.2±12.2	NS	NS
TG (mg/dl)	146.2±89.5	145.8±65.1	147.6±82.0	142.6±55.6	NS	NS
HbA _{1c} (%)	8.8±2.1	8.4±1.8	8.4±1.6	8.0±1.6	NS	NS
HOMA IR	4.12±1.33	4.00±1.56	4.31±1.45	4.19±1.00	NS	NS
hs-CRP (ng/ml)	7.1±4.5	4.6±3.1	7.2±4.0	8.9±4.9	<0.001	0.001
Adiponectin (μg/ml)	8.1±5.8	9.9±6.2	7.4±5.1	6.1±4.4	<0.001	0.004
sCD40L (ng/ml)	8.8±4.9	4.8±4.8	8.4±2.9	10.9±1.8	0.03	0.05
ABI (right leg)	0.77±0.12	0.76±0.18	0.73±0.08	0.78±0.10	NS	NS
ABI (left leg)	0.69±0.17	0.71±0.17	0.67±0.19	0.66±0.11	NS	NS

Differences between the treatment time points were calculated using repeated measures of general linear model.

P, tests of within-subjects contrasts in the group after 6 months of treatment with cilostazol; P*, tests of between-subjects effects.

See Table 1 for abbreviations.

Table 4. Effect of Cilostazol in Non-PAOD Patients

	Cilostazol group		Placebo group		P value	P* value
	Before treatment	After treatment	Before treatment	After treatment		
Age (years)	62.5±10.3		63.3±10.9			NS
Sex (M/F)	21/29		21/29			NS
BMI (kg/m ²)	26.1±2.3	26.4±3.3	25.8±3.1	26.4±5.2	NS	NS
WC (cm)	99.2±11.6	100.2±9.6	97.2±10.9	97.2±9.8	NS	NS
BF (%)	27.0±4.9	27.4±6.6	27.0±6.3	27.6±6.2	NS	NS
TC (mg/dl)	184.1±23.4	188.8±35.8	186.5±33.3	189.9±32.1	NS	NS
LDL (mg/dl)	119.8±30.4	118.7±31.8	112.0±38.3	115.0±32.9	NS	NS
HDL (mg/dl)	49.3±14.9	51.3±11.6	50.5±12.6	50.2±12.7	NS	NS
TG (mg/dl)	155.7±73.5	145.8±65.1	147.6±83.3	142.6±55.6	NS	NS
HbA _{1c} (%)	8.1±2.3	8.4±1.4	8.2±1.0	8.4±1.5	NS	NS
HOMA IR	3.92±1.41	3.87±1.77	4.03±2.10	4.00±1.98	NS	NS
hs-CRP (ng/ml)	2.7±1.5	2.1±3.1	2.4±1.0	2.9±4.9	0.02	0.02
Adiponectin (μg/ml)	9.0±3.7	9.9±6.2	9.1±5.3	8.6±3.0	0.03	0.04
sCD40L (ng/ml)	8.0±3.7	8.3±4.5	8.1±2.9	8.3±1.8	NS	NS
ABI (right leg)	1.15±0.14	1.16±0.23	1.13±0.28	1.15±0.18	NS	NS
ABI (left leg)	1.17±0.33	1.17±0.37	1.15±0.19	1.16±0.21	NS	NS

Differences between the treatment time points were calculated using repeated measures of general linear model.

P, tests of within-subjects contrasts in the group after 6 months of treatment with cilostazol; P*, tests of between-subjects effects.

See Table 1 for abbreviations.

hs-CRP, adiponectin and sCD40L (7.1±4.5 vs 4.6±3.1 ng/ml; 8.1±5.8 vs 9.9±6.2 μg/ml; 8.8±4.9 vs 4.8±4.8 ng/ml tests of within-subjects contrasts, P<0.001, P<0.001 and P=0.03, respectively). Comparing the effects of cilostazol and placebo, we found that patients in the cilostazol group had greater decreases in the levels of TC (190.1±29.4 vs 188.8±35.8 mg/dl) and LDL (119.8±30.4 vs 108.7±33.8 mg/dl)

and greater decreases in the levels of hs-CRP and sCD40L than those in the placebo group (tests of between-subjects effects, P=0.02, P=0.04, P=0.001, P=0.05, respectively). Adiponectin levels were more clearly elevated after 6 months of cilostazol (tests of between-subjects effects, P=0.004).

In the non-PAOD group (Table 4), the changes in the serum levels of hs-CRP and adiponectin were significant

after 6 months of cilostazol (2.7 ± 1.5 vs 2.1 ± 3.1 ng/ml; 9.0 ± 3.7 vs 9.9 ± 6.2 μ g/ml tests of within-subjects contrasts; $P=0.02$ and $P=0.03$, respectively). However, the changes in ABI, BMI, WC, %BF and in the levels of TC, LDL, HDL, TG, HOMA IR, HbA_{1c} and sCD40L were not significant. Comparisons of the effect of cilostazol and placebo on outcome measures revealed that there was a more clear decrease in the serum levels of hs-CRP and increase in adiponectin after 6 months in the group treated with cilostazol than in those treated with the placebo (tests of between-subjects effects, $P=0.02$, $P=0.04$, respectively).

Comparison of Changes in the Parameters of the PAOD and Non-PAOD Groups After Cilostazol Therapy

The changes in the serum levels of adiponectin and sCD40L were more apparent in the PAOD group than in the non-PAOD group (tests of between-subjects effects, $P=0.01$ and $P=0.008$, respectively).

Discussion

PAOD affects 29% of all DM patients aged ≥ 50 years in the United States,⁹ and in Taiwan, approximately 10% of all type 2 DM patients aged ≥ 50 years develop PAOD³ and are also at a high risk of lower-extremity amputation. Chronic inflammation plays an important role in the acceleration of atherosclerosis in DM.⁷ The inflammatory marker, hs-CRP, is strongly associated with the development of PAOD.^{10,11} Adiponectin secreted by adipose cells might regulate nitric oxide and reduce the expression of adhesion molecules in endothelial cells. Several studies have shown that human adiponectin has antidiabetic and antiatherogenic properties, so it is an antiinflammatory marker and may be associated with PAOD.^{12,13} CD40L can activate platelets to form a fresh thrombus and contribute to endothelium dysfunction and inflammation; sCD40L is released from activated platelets within minutes to hours after their activation.¹⁴ Previous studies have reported that individuals with type 1 or type 2 DM have elevated levels of plasma sCD40L,¹⁵ especially those who also have coronary artery disease.¹⁶ In a previous study, circulating levels of CD40 ligand were elevated only in patients with more advanced symptomatic PAOD, but not in type 2 DM patients.¹⁷ In the present study, the ABI was significantly correlated with these pro-inflammatory markers. Our linear regression analyses, which we performed to identify the predictors of PAOD, also revealed the adiponectin level to be 1 of the best predictors of the ABI, besides age.

Cilostazol is able to elevate cyclic AMP levels by inhibiting cyclic nucleotide PDE3 activity and inhibiting platelet aggregation in response to ADP, collagen, epinephrine and arachidonic acid, and has long been used in the treatment of chronic PAOD. A previous report revealed that this drug reduces atherosclerosis by inhibiting superoxide $\text{TNF-}\alpha$ and activating endothelial nitric oxide synthase.¹⁸ A short-term study also showed that cilostazol could reduce 23.6% of the serum level of hs-CRP in type 2 DM patients, although no mention was made of PAOD.¹⁹ This improvement probably occurs as a result of the activation of endothelial nitric oxide synthase.^{20,21} Cilostazol can also stimulate differentiation of fibroblasts into adipocytes and stimulate PPAR- γ transcription, which may contribute to the increased serum adiponectin levels.²² In this study, cilostazol treatment alleviated chronic inflammation (high hs-CRP and low adiponectin) in both groups of DM patients; the increase in adiponectin was

more apparent in the PAOD group.

Cilostazol has antiplatelet and antithrombotic effects. The mechanism of preventing neointimal formation is unclear, but cilostazol may inhibit intima formation, possibly through its inhibition of angiotensin II-forming enzymes or its effect on lipids (15% reduction in plasma TG level and 10% increase in HDL level).^{23–25} In type 2 DM patients, cilostazol improves the post-prandial lipemia.²⁶ Nomura et al demonstrated decreases in the plasma concentrations of soluble adhesion molecules and platelet-derived microparticles in patients with non-insulin dependent DM after oral administration of 150 mg/day of cilostazol for 4 weeks.²⁷ However, they did not measure pro-inflammatory markers or the ABI in their evaluation of the effect of cilostazol. In our study, after 6 months of cilostazol (100 mg/day) therapy, the serum sCD40L levels decreased in type 2 DM patients with PAOD but not in those without PAOD. We also found that the TC and LDL levels, not the TG or HDL level, decreased more significantly in the cilostazol group than in the placebo group. The mechanism underlying cilostazol's sCD40L-lowering effect may not involve the previously mentioned lipid effects, so further studies are needed to clarify how it does this.

Kim et al concluded that there was not an additive effect on aspirin-induced anti-platelet activity or decreasing of sCD40L in patients undergoing primary percutaneous coronary intervention.²⁸ Ahn et al also found that cilostazol could not change the serum level of hs-CRP in patients undergoing coronary stenting after already being treated with aspirin and clopidogrel.²⁹ They found that cilostazol did not have a clear additive effect in patients with acute coronary syndrome or in patients already taking other antiplatelet drug therapy; less than 30% of patients had DM. In our study, after excluding patients with a medical history of coronary artery disease, we found a greater reduction in the serum level of sCD40L in patients with type 2 DM complicated with PAOD than in the non-PAOD DM patients treated with cilostazol. This drug may have more significant anti-atherogenic effects in those who have PAOD than in those who do not. In an animal study, the mRNA expression of PPARs was tissue-specific in DM and may be differently affected by cilostazol,³⁰ which could have different antiplatelet and antiinflammatory effects in different tissues. The ischemic change in the lower extremities may alter the tissue response to cilostazol, so we hypothesize that PAOD-related changes in tissue nutrition may contribute to the more apparent reduction in sCD40L in the DM with PAOD group after cilostazol treatment. Thus, further studies are indicated to clarify this hypothesis.

Study Limitations

According to the Framingham Heart Study, the risk of PAOD can be predicted by age, tobacco use and IR-related metabolic syndromes, including DM, hypercholesterolemia, hypertension and coronary heart disease. However, in our study, the ABI did not correlate with the classical risk factors: BP, lipid profile and HOMA IR. Although office BP may not be a good measure of BP control, many studies, including our own, have not been able to accurately measure home BP or continuously measure 24-h BP, which is a limitation. In the Taiwanese type 2 DM and South Indian populations, a previous study found no association between hypercholesterolemia and the risk of PAOD.³ There is not an appropriately designed lipid-lowering trial in patients with PAOD, so further evaluation is indicated.³¹ The avail-

able lipid profile data we had in this study were insufficient for defining dyslipidemia in the type 2 DM patients. Further evaluation may be needed to determine the level of small-dense LDL or other parameters. Although the HOMA IR model is a widely used tool for evaluating IR, there is a possibility that it could erroneously conclude that patients have poor β -cell function, especially when they are taking high doses of antidiabetic agents and more than 1 type of oral antidiabetic agent.³² Therefore, either HbA_{1c} or HOMA IR may not be an adequate marker for assessing diabetes control. Fluctuation in the blood glucose levels may cause greater damage to blood vessels,^{33,34} so continuous glucose monitoring might be considered when doing future studies. In this study, we also found no significant change in the resting ABI after the 6 months of cilostazol therapy, possibly indicating the need for a longer study. ABI <0.9 may underestimate the prevalence of PAOD in the general elderly population.³⁵ Post-exercise ABI evaluation, contrast angiography or magnetic resonance angiography may be more precise.

Conclusion

PAOD is prevalent in elderly type 2 DM patients. The risk factors of atherogenesis are aging and chronic inflammation, rather than a high HbA_{1c} level or hypercholesterolemia. Adiponectin level may be a good predictor of PAOD. Cilostazol was able to decrease the levels of hs-CRP and sCD40L, and increase that of adiponectin, and then delay the progression of atherogenesis and chronic inflammation in type 2 diabetics, especially those with PAOD.

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