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ORIGINAL ARTICLE

Randomized control trial comparing the effect of cilostazol and aspirin on changes in carotid intima-medial thickness

Sangmo Hong[1,2] · Munsuk Nam[3] · Bertis B. Little[4,5] · Seihyun Paik[6] · Kwanwoo Lee[7] · Jungtaek Woo[8] · Dooman Kim[9] · Jungoo Kang[9] · Minyoung Chun[10] · Yongsoo Park[2,11]

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Abstract

Antiplatelet drugs are effective in preventing recurrence of atherosclerosis in type 2 diabetes (T2D) patients. However, the efficacy and usefulness of antiplatelet drugs on the progression of carotid intima-media thickness (IMT), a marker for evaluating early atherosclerotic vascular disease, has not been analyzed. We conducted a prospective, randomized, open, 36-month trial comparing cilostazol vs. aspirin. A total of 415 T2D patients (age range 38–83 years; 206 females) without macrovascular complications were randomized to either an aspirin (100 mg/day) or cilostazol (200 mg/day) treatment. Patients underwent B-mode ultrasonography annually to assess the IMT and serum levels of inflammatory markers were measured before and after each treatment. Potential confounders were statistically adjusted, and included lipid profiles, HbA1c, body mass index, waist circumference, anti-hypertensive and statin medications. The decrease in mean left, maximum left, mean right and maximum right IMT were significantly greater with cilostazol compared with aspirin (-0.094 ± 0.186 mm vs. 0.006 ± 0.220 mm, $p < 0.001$; -0.080 ± 0.214 mm vs. 0.040 ± 0.264 mm, $p < 0.001$; -0.064 ± 0.183 mm vs. 0.004 ± 0.203 mm, $p = 0.015$; -0.058 ± 0.225 mm vs. 0.023 ± 0.248 mm, $p = 0.022$, respectively). And these differences remained significant after adjustment of potential confounders. Compared with aspirin, cilostazol treatment was associated with significantly increased HDL cholesterol ($p = 0.039$) and 25-hydroxy vitamin D levels ($p = 0.001$). Cilostazol treatment was associated with significantly lowered IMT in T2D patients compared to aspirin, independent of conventional cardiovascular risk factors. Cilostazol may inhibit plaque formation and have beneficial effects on atherosclerosis through vasodilatory and antiplatelet effects.

Keywords Cilostazol · Aspirin · Anti-platelet · Carotid IMT · Type 2 Diabetes · Cardiovascular disease (CVD)

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✉ Yongsoo Park
parkys60@gmail.com

[1] Division of Endocrinology, Department of Internal Medicine, Hallym University Dongtan Sacred Heart Hospital, Gyeonggi-do, South Korea

[2] Department of Internal Medicine and Bioengineering, Hanyang University College of Medicine and Engineering, Seoul, South Korea

[3] Department of Internal Medicine, Inha University School of Medicine, Incheon, South Korea

[4] Bertis B. Little, Department of Health Management and Systems Sciences, University of Louisville, Louisville, KY, USA

[5] Division of Cardiology, Medical Service, Dallas VA Medical Center, Dallas, TX, USA

[6] Division of Endocrinology and Metabolism, Department of Internal Medicine, Korea University College of Medicine, Seoul, South Korea

[7] Department of Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, South Korea

[8] Department of Endocrinology and Metabolism, Kyung Hee University School of Medicine, Seoul, South Korea

[9] Department of Endocrinology and Metabolism, Hallym University School of Medicine, Seoul, South Korea

[10] Department of Global Medical Science, Sungshin Women's University, Seoul, South Korea

[11] Health Insurance Review and Assessment Service, Uijeongbu, South Korea

Introduction

Type 2 diabetes (T2D) is associated with a high rate of atherosclerotic cardiovascular disease (CVD). Patients with T2D usually present with additional cardiovascular risk factors (e.g., hypertension, dyslipidemia, obesity, and hyperglycemia) [1]. A number of studies have revealed the efficacy of modifying individual cardiovascular risk factors in patients with T2D. Antiplatelet drugs are also effective in preventing CVD events in T2D patients with clinical CVD (history of acute coronary syndrome, myocardial infarction, stroke, transient ischemic attack, or peripheral arterial disease; secondary prevention) [2]. Aspirin is also recommended as a primary prevention strategy in diabetes patients with increased cardiovascular risk (10-year risk > 10%) [1, 3], but there was no benefit of aspirin for primary prevention in patients with T2D in two randomized controlled trials (RCTs) and meta-analysis [4–6]. Several lines of evidence suggest that the efficacy of aspirin in patients with T2D is substantially lower than in individuals without T2D [3]. The benefit of other antiplatelet drugs in the primary prevention of CVD among T2D patients remains to be investigated.

Cilostazol is a reversible, selective inhibitor of the phosphodiesterase 3, which is responsible for the hydrolysis of cAMP and cGMP and is present both in platelets and smooth muscle cells (SMCs), and leads to inhibit platelet activation, smooth muscle constriction and proliferation [7]. Experimental studies showed that cilostazol, an antiplatelet agent, suppressed proinflammatory markers and superoxide production, protected vessel endothelium, inhibited proliferation of vascular SMCs and formation of foam cells in macrophages [8–11]. Although some studies have reported that cilostazol may be effective in preventing cardiovascular events in patients with T2D, the number of such studies is still relatively small or has been done for short observation period [7, 12]. Therefore, the role of cilostazol in the primary prevention of CVD among T2D patients remains unclear.

Carotid artery intima-media thickness (IMT) is a surrogate marker for evaluating atherosclerotic vascular disease [13]. Carotid IMT and plaques are strongly correlated with development of CVD [14, 15]. Studies of the association between CVD development and carotid IMT in Asian T2D patients are limited. The purpose of the present investigation was to test the hypothesis that cilostazol significantly reduces carotid IMT of T2D patients compared to aspirin.

Materials and methods

Study design and participants

The Cilostazol versus Aspirin for Primary Prevention of Atherosclerotic Events with Korean T2D patients (CAPPA) study is an investigator-initiated, randomized, open, 36-month, multicenter trial. Among the patients with T2D who joined the Korean National Diabetes Program (KNDP) cohort study [16, 17], those who did not have evident CVDs (cerebrovascular disease, coronary artery disease or peripheral vascular disease), but had multiple risk factors, were invited to participate in this trial. The details of inclusion and exclusion criteria were listed in Supplement Table 1. A total of 420 patients were recruited from the KNDP and randomized in this study; 210 patients in the aspirin group and 210 patients in the cilostazol group. Five patients in the cilostazol group were excluded from the study because three missed the baseline IMT measurement appointments and two patients withdrew informed consent. Per-protocol analysis (PPA) was performed in 308 patients, including 164 and 144 patients in the aspirin and cilostazol groups, respectively, who completed the 3 years follow-up (Fig. 1). Table 1 shows the baseline characteristics of these 415 subjects. Supplement Table 2 shows the baseline characteristics of these 308 patients. The study protocol was approved by the ethics review board at each participating site and has been registered at ClinicalTrials.gov (NCT00886574).

Randomization and procedure

At screening visit, trained examiners interviewed all patients using a questionnaire that included items on health status including past medical history of CVD, tobacco smoking, medications for T2D, hypertension and statins. Patients who are qualified according to the inclusion/exclusion criteria were registered through the study website to the central office and were randomly allocated (1:1) to cilostazol or aspirin groups (aspirin, 100 mg/day or cilostazol, 200 mg/day) by a computer-generated randomized method. After signing the informed consent, patients were advised of lifestyle modifications by diabetes education specialists, who counseled them according to the ADA standards of medical care in diabetes [1].

Each patient was followed for 3 years in seven outpatient visits at 0, 6, 12, 18, 24, 30, and 36 months. Other antiplatelet agents, anticoagulants, thrombolytic agents, vasoactive agents, and hemorheological agents were prohibited during the study period. Anthropometric parameters (height, weight and waist circumference), blood

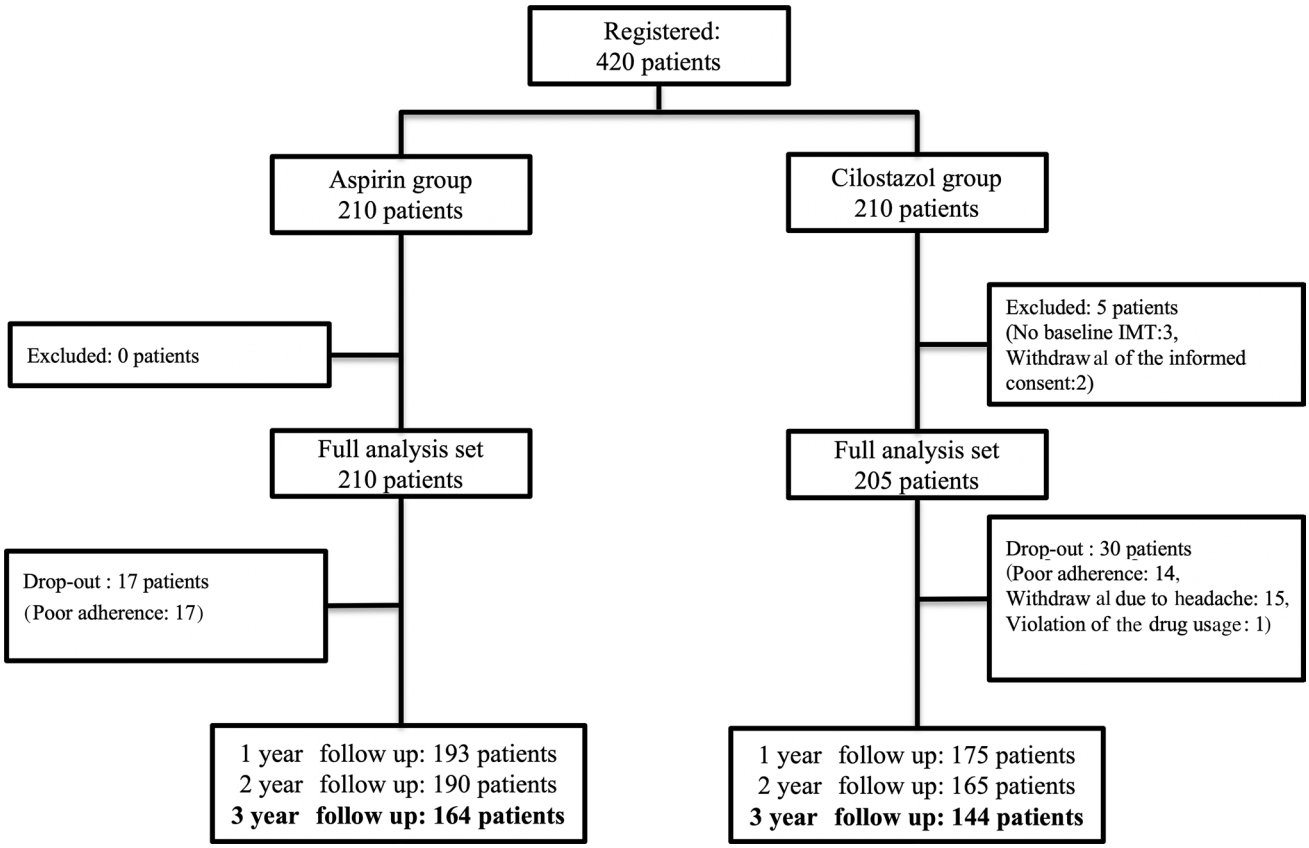

Fig 1. Patient disposition of the current study. A total of 420 subjects were screened, with respective 210 and another 210 subjects randomized to the aspirin group and the cilostazol group. Of the randomized 420 subjects, 2 subjects in the cilostazol group were excluded due to early withdrawal of the informed consents and 3 more subjects in the cilostazol group were excluded because of no baseline IMT. As a result, it turned out that 415 subjects were included in the full analysis set. Among those, another 17 subjects in the aspiring group were excluded before 1 year follow-up due to poor adherence, while another 30 subjects in the cilostazol group were also excluded with similar reasons (poor adherence in 14 subjects; consent withdraw due to persistent headache in 15 subjects; and use of prohibited drugs in 1 subject) before 1 year has passed. Among the remaining 193 subjects in the aspirin group and 175 subjects in the cilostazol group, 164 and 144 subjects fulfilled the required annual follow-up for 3 years and were analyzed for efficacy and safety during and after 3 years of respective treatment. Among 415 subjects who received at least one dose of aspirin or cilostazol after randomization and had a baseline carotid IMT measurement for efficacy assessment, 164 subjects in the aspirin group and 144 subjects in the cilostazol group fulfilled the 3 year requirement of the treatment and follow-ups and were assessed for efficacy (per protocol set)

pressure, pulse rate, fasting plasma glucose (FPG), HbA1c, fundus photography, 12-lead ECG and CVD events were recorded at baseline and annually thereafter. Serum levels of 25-hydroxy vitamin D (vitamin D), vascular cellular adhesion molecule-1 (vCAM-1), high sensitivity C-reactive protein (hsCRP), monocyte chemotactic protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-α) were measured at beginning and end of the study. Serum vitamin D and CRP levels were measured using a COBAS Integra 700 analyzer (Roche Diagnostics, Mannheim, Germany) with electrochemiluminescence immunoassays according to the manufacturer's specifications. Serum levels of vCAM-1, MCP-1 and TNF-α were measured using a MILLIPLEX™ Human Cytokine/Chemokine panel (Millipore, Billerica, MA). Intra- and interassay coefficients of variation were 4.5 and 8.5% for vCAM-1, 6.1 and 12.0% for MCP-1 and 10.5 and 15.9% for TNF-α, respectively.

Carotid artery IMT measurement

Patients underwent B-mode ultrasonography with a high-frequency linear transducer (7.5- to 12-MHz) to assess the IMT of the far walls of both CCAs at baseline and annually thereafter. Ultrasonographic scans of the CCAs were performed by the unchanged, well-trained, protocol-adherent sonographer with the same ultrasound system to minimize intra-observer variability. The IMT was measured as the distance between the leading edges of the lumen–intima and media–adventitia ultrasound interfaces on the far wall of the CCA using digital calipers on an image. A 1 cm long image

Table 1 Baseline characteristics of the study participants

	Aspirin group FAS ($n=210$)	Cilostazol group FAS ($n=205$)	p value
Age (years)	59.5 ± 9.4	59.7 ± 9.0	0.986
Sex (Men, %)	109 (51.9%)	100 (48.7%)	0.526
Body mass index (kg/m^2)	25.5 ± 3.3	25.3 ± 2.8	0.566
Waist circumference (cm)	89.5 ± 8.2	88.2 ± 7.9	0.129
Current smoker (%)	87 (41.4%)	89 (43.4%)	0.692
Systolic blood pressure (mmHg)	128 ± 14	127 ± 13	0.279
Diastolic blood pressure (mmHg)	78 ± 8	78 ± 9	0.698
Hypertension (≥ 140/90 or medication)	143 (68.1%)	145 (70.7%)	0.595
Fasting blood glucose (mg/dl)	137 ± 44	136 ± 38	0.727
HbA1c (%)	7.3 ± 1.1	7.5 ± 1.2	0.114
Total cholesterol (mg/dl)	175 ± 36	174 ± 34	0.686
LDL-cholesterol (mg/dl)	95 ± 33	94 ± 31	0.693
HDL-cholesterol (mg/dl)	50 ± 12	50 ± 13	0.860
Triglyceride (mg/dl)	155 ± 86	162 ± 154	0.724
Hypercholesterolemia (LDL-c > 130 mg/dL or statin medication)	126 (60.3%)	108 (52.7%)	0.137
Hypertriglyceridemia (> 200 mg/dL)	81 (38.6%)	89 (43.4%)	0.320
Serum creatinine (mg/dl)	0.87 ± 0.26	0.86 ± 0.37	0.418
eGFR (ml/min/1.73m^2)	87 ± 30	86.1 ± 32.8	0.983
Microalbuminuria	57 (27.1%)	59 (28.7%)	0.743
Macroalbuminuria	40 (19.5%)	38 (18.5%)	0.900
eGFR < 60 ml/min/1.73m^2	39 (18.9%)	39 (19.0%)	0.876
Retinopathy	63 (30.0%)	71 (34.6%)	0.345
Macular edema	4 (1.9%)	5 (2.4%)	0.749
CCA-mean IMT (mm)			
Left	0.932 ± 0.204	0.933 ± 0.209	0.935
Right	0.918 ± 0.188	0.924 ± 0.190	0.765
CCA-maximum IMT (mm)			
Left	1.096 ± 0.250	1.093 ± 0.256	0.912
Right	1.092 ± 0.282	1.100 ± 0.255	0.758

CCA common carotid artery, *eGFR* estimated glomerular filtration rate, *FAS* full analysis set, *HDL* high-density lipoprotein, *IMT* intima media thickness, *LDL* low-density lipoprotein

was obtained on the far wall of CCA 10 mm to 20 mm apart from the bifurcation of internal carotid and external carotid. All ultrasound scans were stored electronically. And the mean and maximum IMTs were measured in a central office by a reader who is blinded to each patient's treatment arm randomization with automated digital edge-detection software (Intimascope; MediaCross, Tokyo, Japan). The mean IMT was the average thickness of the wall, and the maximum IMT was the greatest thickness of the wall, including plaque lesions. An atherosclerotic plaque was confirmed if its thickness was ≥ 50% greater than the surrounding IMT, IMT was > 1.5 mm, or there was a region > 5 mm thicker than the surrounding IMT. To measure reader reproducibility, IMT for the first 122 subjects was measured repeatedly, and the results indicated that there was a 2.11% inter-observer coefficient of variation (CV) and 2.06% intra-observer CV. To minimize inter-measurer errors**,** workshops were conducted for ultrasound measurers from all participating institutions before study initiation and annually during the study, and inter-measurer reproducibility was measured for reference. During the entire period, intra-class correlation for ultrasound measurers was 0.97 to 0.99 for mean IMT.

Primary and secondary outcome

The primary end-points were the changes in mean and maximum IMTs of both CCAs over the course of 3 years. The secondary end-point was development of major adverse cardiovascular events (MACE), as defined below. The safety profile of the medications administered including major bleeding events, hemorrhagic stroke, and death due to any cause during the study was also established. MACE was

defined as a composite end-point of first-ever acute myocardial infarction, coronary-artery bypass graft, percutaneous coronary intervention, ischemic stroke, hemorrhagic stroke, or death due to any cardiovascular event. The individual cardiovascular events were defined according to the criteria set by the WHO/MONICA (World Health Organization/Multinational Monitoring of Trends and Determinants in Cardiovascular Disease) project [18].

Sample size

For the primary end-point on changes of mean IMT (ΔIMT_{mean}), calculation of the required sample size was carried out according to the previous study results in which there was no difference of ΔIMT_{mean} between the cilostazol group and untreated group with a standard deviation (SD) of 0.16 mm [8], while there was a 0.10 mm difference of ΔIMT_{mean} between the aspirin group and untreated group with a SD of 0.03 mm during a 3-year follow-up [19]. We assumed that there would be a 0.10 mm difference in ΔIMT_{mean} between the aspirin group and the cilostazol group in 3 years. The larger published SD (0.16 mm) was used to obtain a conservative estimate of power, resulting in planned sample size of 92 patients (46 patients in each arm) to achieve 80% power (1-β) and an α of 0.05 (two tailed). The present study has sufficient power to detect a difference of 0.05 mm in IMT. An additional primary end-point is the difference in the ΔIMT_{max}. Two hundred subjects per group were enrolled to account for worst case scenario of high attrition from the study over a 3-year period because significant loss to follow-up can affect longitudinal study success.

Statistical analysis

Baseline characteristics are presented as means and SDs for continuous variables, and percentages for proportions of categorical variables. Medians and ranges are shown for the values of CRP because of the non-normal distribution. Differences in the baseline characteristics between different treatment groups were compared using the χ^2 test and t test for categorical and continuous variables, respectively. A one-way repeated measures analysis of variance (ANOVA) was used to analyze within-group changes over time for 3 years. The primary end-points between two treatment arms were compared using the two-way repeated measures ANOVA and the repeated measures analysis of covariance (ANCOVA) with adjusting the covariant. Friedman's test was used for the comparison of the CRP levels across time. The secondary end-point was 'time-to-event' of MACE. Safety comparisons were assessed using Chi-square for proportions. All p values were two-sided, and the significance level was 0.05. Bonferroni adjustment for multiple comparisons was used. Bootstrapping (re-sampling) analysis was used for sub-group analyses. To confirm the robustness of better effect of cilostazol over aspirin, a sensitivity analysis using generalized linear model (GLM) of mean right, maximal right, mean left and maximal left IMT data separately was performed. To confirm and highlight consistency in effect among different subgroups, forest plots of left and right IMT means were depicted according to the treatment group. All statistical analyses were performed using IBM SPSS software (IBM SPSS version 24.0, Chicago, Illinois USA). Power analysis was done using Power and Precision v.4.1.0 software (www.Power-Analysis.com, Dr. Michael Borenstein, Englewood, NJ USA).

Results

There were no statistically significant differences in baseline demographic or clinical characteristics (including baseline IMT of left/right mean or maximum) between patients randomized to aspirin and cilostazol (Table 1). In the aspirin group, whereas mean IMT of left and right CCA (ΔIMT_{mean}: 0.008 ± 0.222 mm, $p = 0.388$ and 0.000 ± 0.204 mm, $p = 0.538$, respectively) and maximum IMT of right CCA (ΔIMT_{max}: 0.017 ± 0.248 mm, $p = 0.128$) revealed no longitudinal changes over the study time, left ΔIMT_{max} increased over the study period (ΔIMT_{max}: 0.042 ± 0.264 mm, $p = 0.037$). In contrast, the cilostazol group had statistically significant decreases in all measures of IMT during 3 years of treatment (-0.089 ± 0.183 mm, $p < 0.001$ for left ΔIMT_{mean}; -0.069 ± 0.216 mm, $p < 0.001$ for left ΔIMT_{max}; -0.063 ± 0.183 mm, $p < 0.001$ for right ΔIMT_{mean}; -0.059 ± 0.226 mm, $p = 0.001$ for right ΔIMT_{max}) (Fig. 2). The reduction in IMT in the cilostazol group was significantly superior compared to the aspirin group in two-way repeated measures ANOVA ($p < 0.001$ for left ΔIMT_{mean}, $p = 0.001$ for right ΔIMT_{mean}, $p < 0.001$ for left ΔIMT_{max}, and $p = 0.011$ for right ΔIMT_{max}) (Table 2). Intergroup differences in the ΔIMT over 3 years between cilostazol and aspirin arms of the study were significant for all IMT parameters after adjustment for potential confounders [age, sex, body mass index (BMI), waist circumference, HbA1c, low-density lipoprotein (LDL) cholesterol, systolic and diastolic pressure, smoking, treatment of antihypertensive medication and duration of statin usage] in an ANCOVA model ($p < 0.005$) (Table 2).

No statistically significant differences in clinical characteristics and laboratory measurements were found between the cilostazol and aspirin groups after 3 years of treatment, except HDL cholesterol and vitamin D levels (Table 3). HDL cholesterol level increased in the cilostazol group (50 ± 13 mg/dl to 54 ± 16 mg/dl) compared to aspirin (50 ± 13 mg/dl to 51 ± 15 mg/dl, $p = 0.039$) after 3 years. Vitamin D levels also increased in the cilostazol

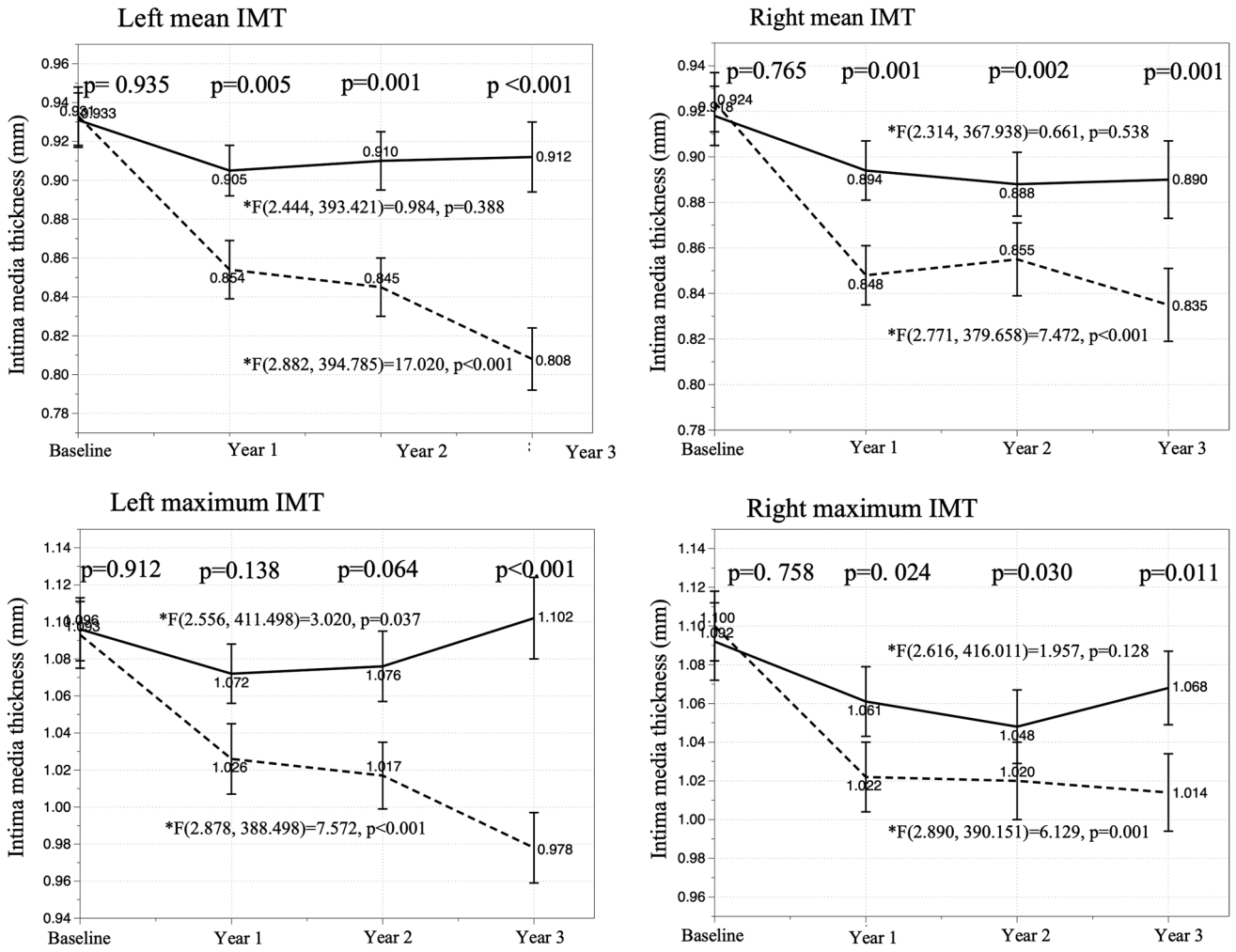

Fig 2. Changes of IMTs between two different treatments during the study period. Solid lines denote for the IMT values of the aspirin treatment, while dotted lines denote for those of the cilostazol treatment group. Individual mean IMT value and standard error was shown at each year of the treatment. *P* values above lines are resulted from comparing the changes of IMTs between two treatments using repeated measure ANOVA. And *F* ratios and *p* values below each line were calculated as the effect of time within the treatment group by one-way repeated measure ANOVA

group (13.2 ± 5.4 ng/dl to 16.6 ± 7.1 ng/dl, within group: $p = 0.005$, between groups: $p < 0.001$) compared to aspirin (15.8 ± 6.8 ng/dl to 14.2 ± 6.3 ng/dl). Cilostazol treatment tended to decrease vCAM-1 levels over time (1297.0 ± 284.2 to 1174.1 ± 271.2 ng/dl), but not significantly, compared to aspirin (1288.7 ± 285.6 to 1381.5 ± 250.2 ng/dl) ($p = 0.09$). Similarly, hsCRP levels tended to improve after cilostazol treatment ($p = 0.10$); by bootstrapping analysis the difference was significant ($p < 0.001$). Other biochemical markers (lipid profiles, MCP-1 and TNF-α) did not differ between the two groups. Interestingly, the proportion of patient statin use over the 3 years of the study increased significantly ($p < 0.05$) more in the aspirin (27.4% to 54.9%) compared to cilostazol (27.8% to 40.3%) groups.

No significant difference in the incidence of MACE, our secondary end-point between aspirin and cilostazol groups (2.38% vs. 2.93%, respectively) was found (Table 4). The frequency of other adverse outcomes, except headache, was not significantly different between cilostazol and aspirin groups during this trial either. Cilostazol induced headache was more frequent than aspirin (12.6% vs. 0.48%, $p < 0.001$, respectively), resulting in a higher dropout rate in the cilostazol group during the first year after the enrollment.

Discussion

The findings of this prospective, 36-month RCT showed that cilostazol significantly inhibited the progression of IMT more than aspirin in patients with T2D. The decrease in mean and maximum IMT of left and right CCA was greater with cilostazol compared with aspirin for 3 years. Our

Table 2 Changes in carotid artery intima media thickness (IMT) during 3 years of different treatment

	Aspirin group	Cilostazol group	*P* (interaction between treatment groups and time)+		
			Unadjusted	Model 1	Model 2
Left mean IMT					
Baseline	0.932 ± 0.204	0.933 ± 0.209	0.935		
Year 1	0.905 ± 0.185†	0.854 ± 0.213*			
Year 2	0.910 ± 0.202	0.845 ± 0.191*			
Year 3	0.912 ± 0.229	0.808 ± 0.198*			
Mean change (ΔIMT), year 1	− 0.025 ± 0.150	− 0.069 ± 0.161	0.005	0.004	0.004
Mean change (ΔIMT), year 2	− 0.006 ± 0.182	− 0.066 ± 0.182	0.001	0.001	0.001
Mean change (ΔIMT), year 3	0.008 ± 0.222	− 0.089 ± 0.183	< 0.001	< 0.001	< 0.001
Left maximum IMT					
Baseline	1.096 ± 0.250	1.093 ± 0.256	0.912		
Year 1	1.072 ± 0.237	1.026 ± 0.259*			
Year 2	1.076 ± 0.257	1.017 ± 0.230†			
Year 3	1.102 ± 0.286†	0.978 ± 0.225*			
Mean change (ΔIMT), year 1	− 0.021 ± 0.186	− 0.051 ± 0.205	0.138	0.124	0.138
Mean change (ΔIMT), year 2	0.000 ± 0.219	− 0.051 ± 0.228	0.064	0.052	0.053
Mean change (ΔIMT), year 3	0.042 ± 0.264	− 0.069 ± 0.216	< 0.001	< 0.001	< 0.001
Right mean IMT					
Baseline	0.918 ± 0.188	0.924 ± 0.190	0.765		
Year 1	0.894 ± 0.183†	0.848 ± 0.182*			
Year 2	0.888 ± 0.192	0.855 ± 0.208*			
Year 3	0.890 ± 0.213	0.835 ± 0.191*			
Mean change (ΔIMT), year 1	− 0.023 ± 0.124	− 0.070 ± 0.165	0.001	0.001	< 0.001
Mean change (ΔIMT), year 2	− 0.016 ± 0.162	− 0.055 ± 0.209	0.002	0.002	0.001
Mean change (ΔIMT), year 3	0.000 ± 0.204	− 0.063 ± 0.183	0.001	0.002	0.001
Right maximum IMT					
Baseline	1.092 ± 0.282	1.100 ± 0.255	0.758		
Year 1	1.061 ± 0.251†	1.022 ± 0.245*			
Year 2	1.048 ± 0.258	1.020 ± 0.252*			
Year 3	1.068 ± 0.243	1.014 ± 0.243†			
Mean change (ΔIMT), year 1	− 0.028 ± 0.191	− 0.073 ± 0.199	0.024	0.021	0.006
Mean change (ΔIMT), year 2	− 0.022 ± 0.243	− 0.068 ± 0.242	0.030	0.026	0.010
Mean change (ΔIMT), year 3	0.017 ± 0.270	− 0.059 ± 0.226	0.011	0.009	0.002

Model 1 Adjusted by age, sex

Model 2 Adjusted by age, sex, BMI, waist circumference, HbA1c, LDL cholesterol, systolic and diastolic pressure, smoking, treatment of antihypertensive medication and duration of statin usage

*Significant difference compared with the baseline IMT ($p < 0.001$)

†Significant difference compared with the baseline IMT ($p < 0.01$)

+*p* values are derived using the two-way repeated-measures ANOVA and the repeated measures analysis of covariance (ANCOVA) with adjusting the following covariants

results persisted after adjustment for possible confounders (Table 2) and subgroup analysis by age, sex and statin usage (Supplement Fig. 1). In a sensitivity analysis using GLM, we also confirmed robustness of better effect of cilostazol over aspirin (Supplement Table 3). In a prior study, cilostazol was significantly associated with slowed progression of atherosclerosis measured by IMT in T2D patients with peripheral vascular disease [11]. Overall, data from this study and previous studies suggest that cilostazol may reduce IMT over time and slow the progression of luminal stenosis regardless of the severity of atherosclerosis. Therefore, cilostazol may be a viable alternative to reduce the risk of CVD in T2D patients.

The number of Asian patients with T2D is dramatically increasing, as will the attendant prevalence of diabetic arteriosclerosis [20]. Incidence of CVD events in subjects with

Table 3 Changes in clinical characteristics between the two groups during 3 years

	Aspirin group			Cilostazol group			*P* value†
	Baseline	3 year follow up	*P* value*	Baseline	3 year follow up	*P* value*	
Body mass index (kg/m^2)	25.3 ± 3.2	25.3 ± 3.1	0.746	25.0 ± 2.7	24.6 ± 2.6	0.036	0.194
Waist circumference (cm)	89.0 ± 8.1	89.0 ± 8.3	0.998	87.1 ± 7.2	87.6 ± 7.5	0.431	0.547
Systolic blood pressure (mmHg)	128 ± 14	127 ± 13	0.515	126 ± 14	124 ± 14	0.191	0.582
Diastolic blood pressure (mmHg)	77 ± 9	76 ± 9	0.039	78 ± 8	75 ± 9	0.010	0.355
HbA1c (%)	7.4 ± 1.2	7.2 ± 1.2	0.256	7.3 ± 1.1	7.1 ± 0.9	0.222	0.884
Total cholesterol (mg/dl)	175 ± 34	163 ± 34	< 0.001	172 ± 33	166 ± 32	0.134	0.175
LDL-cholesterol (mg/dl)	95 ± 34	85 ± 28	0.003	93 ± 30	87 ± 25	0.050	0.312
HDL-cholesterol (mg/dl)	50 ± 13	51 ± 15	0.102	50 ± 13	54 ± 16	< 0.001	0.039
Triglyceride (mgl/dl)	151 ± 80	141 ± 87	0.117	147 ± 86	126 ± 101	0.011	0.437
Serum creatinine (mg/dl)	0.86 ± 0.26	0.89 ± 0.29	0.074	0.85 ± 0.25	0.89 ± 0.28	0.003	0.384
eGFR (ml/min/1.73m^2)	87.8 ± 29.3	85.7 ± 26.3	0.246	85.7 ± 35.8	81.2 ± 25.8	0.052	0.442
25(OH)vitamin D (ng/ml)	15.8 ± 6.8	14.2 ± 6.3	0.100	13.2 ± 5.4	16.6 ± 7.1	0.005	0.001
vCAM-1 (ng/ml)	1288.7 ± 285.6	1381.5 ± 250.2	0.118	1297.0 ± 284.2	1174.1 ± 271.2	0.123	0.09
hsCRP (mg/L)**	1.8 (0.1–3.4)	1.7 (0.1–3.5)	0.532	1.9 (0.1–3.7)	1.4 (0.1–3.2)	0.067	0.10
Statin medication (%)	27.4%	54.9%	< 0.001	27.8%	40.3%	< 0.001	0.012
Hypertension medication (%)	53.5%	68.8%	< 0.001	57.1%	68.9%	< 0.001	0.695

eGFR estimated glomerular filtration rate, *HDL* high-density lipoprotein, *hsCRP* high sensitivity C-reactive protein, *LDL* low-density lipoprotein

*p values were derived from the one-way repeated measures ANOVA within the medication group

**Median (range), p values were derived from the nonparametric Friedman's test

†p values were derived from the two-way repeated measures ANOVA between the medication groups

Table 4 Adverse events, newly developed cardiovascular diseases, and deaths during patient follow-ups

	Aspirin		Cilostazol		*P* value
	N = 210	%	*N* = 205	%	
Completed 3 year follow up study	164	78.10	144	70.24	–
Completed 2 year follow up study	190	90.48	165	80.49	–
Deaths	1[a]	0.48	1[b]	0.49	0.986
Dropped out	17	8.10	30	14.63	0.038
Nonmedical reasons	17	8.10	29	14.15	0.052
Violation of drug use	0	0.00	1	0.49	–
Cardiovascular disease development	5	2.38	6	2.93	0.730
Coronary artery disease	3	1.43	2	0.98	0.674
Cerebral infarction	0	0.00	1	0.49	–
Cerebral hemorrhage	2	0.95	2	0.98	0.981
Peripheral vascular disease	0	0.00	1	0.49	–
Side effects					
Headache	1	0.48	26	12.6	< 0.001
Gastrointestinal discomforts	6	2.86	10	4.88	0.291
Gastrointestinal bleeding	1	0.48	0	0.00	–

[a]Cerebral hemorrhage after a car accident

[b]Low extremity thrombophlebitis; Excessive bleeding after treatment with a thrombolytic agent

T2D is known to be nearly the same as in nondiabetic subjects with prior myocardial infarction during a 7-year follow-up [21]. Therefore, the treatment strategy for CVD in the general population may be applied to patients with T2D for the primary prevention of CVD. However, the efficacy of antiplatelet drugs for primary prevention of CVD in patients with T2D has not been sufficiently demonstrated. A recent study indicated that aspirin had no statistically significant effect in primary prevention of CVD in patients with T2D [6]. Although all patients enrolled in this trial had low

grade of carotid stenosis at the beginning and end of the trial, however, cilostazol decreases all measurements of IMT consistently. In the aspirin group, no significant change in left IMT_{mean}, right IMT_{mean} and right IMT_{max} was observed during the 3-year duration of the study and left IMT_{max} was increased after 3 years. By the way, the frequency of MACE was not significantly different between cilostazol and aspirin groups during this trial. 3 years might be not sufficient enough to compare the 'time-to-event' of MACE, secondary end-point, although cilostazol may gradually slow the development of CVD in patients with T2D.

Compared with aspirin, the effect of cilostazol was significantly greater in left ΔIMT_{mean} and left ΔIMT_{max} compared to right ΔIMT_{mean} and right ΔIMT_{max}. In general, the right IMT had good correlation with left IMTs. In our study, the Pearson correlation coefficient between right and left ΔIMT_{max} after 3 years in all patients was 0.638 ($p < 0.001$), while that between right and left ΔIMT_{mean} after 3 years was 0.711 ($p < 0.001$). In the cilostazol arm of the study, the correlations between right and left ΔIMT_{max} was 0.661 ($p < 0.001$), while those between ΔIMT_{mean} was 0.756 ($p < 0.001$). In contrast, in the aspirin arm of the study, the correlations between right and left ΔIMT_{max} was 0.622 ($p < 0.001$), while those between right and left ΔIMT_{mean} was 0.671 ($p < 0.001$). It is not clear whether the IMT in both CCA are really equal because several studies have shown a greater predictive value for an increase of IMT in the left CCA compared to right CCA [22–26]. A theoretical explanation is that increased shear stress forces in the left CCA contribute to this difference [24, 25]. Left and the right IMT differences may also vary with the stage of CVD progression [22, 26]. Potential confounding effects of age, gender, blood lipid levels, blood glucose level, and other risk factors (BMI, waist circumference, HbA1c, treatment of antihypertensive medication and statin medications) that may affect the left vs. right IMT differences were controlled as covariates. In a prior investigation, no significant differences were observed between left and right IMT in the non-CVD group, but the difference was significant in the CVD group [22]. Left and right CCA differences observed in our patients, which are more evident in the aspirin treated group after 3 years are apparently a combined effect of various atherosclerotic risk factors and potential differences between the shear forces between the two carotid arteries [26]. A more detailed further study is required to understand differences observed between left and right intima media complex, especially with regard to differentiation between normal and the CVD groups.

Accumulated evidence supports the use of cilostazol as the drug of choice for patients with peripheral arterial disease, which shares a common pathophysiology with other CVDs [10, 11, 19, 27]. However, the long-term effects of cilostazol in preventing CVDs in patients with T2D need further investigation. Moreover, the true mechanism for the apparent IMT improvement is unknown. 3 years treatment of cilostazol significantly increased serum HDL cholesterol and 25-hydroxy vitamin D levels compared with aspirin. When we consider the similarly documented effect of cilostazol [11, 28, 29], the favorable effect of cilostazol on IMT might, therefore, in part, be resulted from the accompanying improvement of these. Lower level of serum 25-hydroxy vitamin D levels has been reported to be associated with vascular endothelial dysfunction in T2D patients [30, 31]. In other study with healthy human, vitamin D deficiency is associated with increased circulating inflammatory proteins [32–34], which are associated with increased CVD risk. And vitamin D deficiency is known to be an independent predictor of CVD and all-cause mortality [35, 36]. Although the beneficial effect of cilostazol on IMT might be ascribed to the concomitant improvement of 25-hydroxy vitamin D levels, however, the improvements of 25-hydroxy vitamin D concentration alone could not explain the real changes of IMT after treatment. Anti-inflammatory and vasodilatory effects of cilostazol treatment may be pronounced in the context of increased serum vitamin D status, which also needs to be elucidated. Improved hsCRP and vCAM-1 levels might be associated with decreased IMT after cilostazol treatment compared with aspirin, which was found to be not true either. Further studies are absolutely needed to elucidate the differential changes of IMT after cilostazol treatment compared with aspirin.

Independent vasodilatory and antiplatelet effects of cilostazol are shown in several independent studies [8–11]. Headache associated with cilostazol apparently due to vasodilation has been reported as a side-effect and was the reason a number of patients withdrew informed consents or were lost to follow-up. Notwithstanding, this phosphodiesterase inhibitor may have beneficial effects on carotid lumen thickness. The etiology of the effect is not clear because the drug has an inhibitory effect on plaque accumulation, anti-inflammatory properties, and is a well-known vasodilatory and antiplatelet agent. Importantly, cilostazol activates endothelial nitric oxide synthase (eNOS) by increasing cyclic AMP [8, 10, 11]. Of these possible mechanisms, anti-inflammatory, vasodilatory, and eNOS effects are likely the primary actions of cilostazol on IMT because plaque regression has not been associated with the study drug.

Our study has several strengths. T2D patients were recruited from a well-defined Korean cohort (the KNDP cohort) [16, 17], in which the patients' diagnoses and MACE evaluation were determined by specialists. Detailed changes in various parameters known to affect CVD development such as age, duration of diabetes, smoking status, medication status and biochemical variables as well as drug side effects could be reported. Secondly, the present study is a prospective, 36 month-RCT comparing the effects of

cilostazol vs. aspirin in patients with T2D. Several studies show that cilostazol is associated with slowed progression of atherosclerosis, but it remains unclear whether cilostazol actually has a beneficial impact on the progression of carotid atherosclerosis in patients with T2D at large. A prospective RCT for sufficient study period can answer this important question contrary to the prior short trials with a small number of subjects [8–11]. Finally, to account for attrition from the study over a 3-year period we conducted an RCT in a representative T2D sample > 2 times the size estimated by power analysis. The PPA set was also sufficiently large to analyze the primary outcome differences and sub-analyses while maintaining adequate statistical power.

Limitations of our study included the inevitable bias of an open-label trial. We chose a prospective, randomized, open-label and blinded end-point study design because it could obtain the greatest compliance and follow-up. An additional limitation is that crossover between the two treatment arms was not allowed in this design. Importantly, differences in dropout rate between the two study groups were marked. Secondly, our study population includes only Asian T2D patients without any history of macrovascular complications, but with multiple risk factors. However, evidence suggests that ethnic variation in response to the antiplatelet regimens we studied and IMT seems consistent across populations. Western populations with and without T2D tend to have more higher IMT values, demonstrating differences in drug effects such as aspirin and cilostazol. If we performed a similar trial in Western populations, sample sizes to confirm the differential treatment effect of cilostazol over aspirin may be smaller to achieve desired power (80%). Third, the incidence of CVDs was not different between the two groups, although the 3-year changes of IMT were significantly improved in the cilostazol treatment groups. This trial was developed primarily to assess the relationship between changes in carotid IMT with cilostazol and aspirin treatment groups, and secondarily, the development of CVD over 3 years in the two treatment arms. Therefore, to detect differences in the development of CVDs between the two treatment groups and to estimate the role of cilostazol in primary prevention, it may be necessary to follow patients with more atherosclerotic risk factors for longer than 3 years.

In conclusion, a statistically significant association between cilostazol use and decreased CCA IMT was found after adjustment for age, gender, blood lipid and glucose levels, BMI, waist circumference, HbA1c, treatment of antihypertensive medication and statin medication use. The association of cilostazol treatment with significantly lower carotid IMT in T2D patients compared with aspirin suggests the drug might be an alternative option for the treatment of CCA impairment in T2D. The effect of cilostazol was independent of other cardiovascular risk factors, including high HDL cholesterol and low vitamin D levels. Cilostazol treatment was associated with a reduction in pro-inflammatory cytokines, CRP and adhesion molecules. This drug may have beneficial effects on atherosclerosis by virtue of its anti-inflammatory, plaque inhibiting, vasodilatory and antiplatelet properties.

Compliance with ethical standards

Conflict of interest No potential conflict of interest relevant to this article was reported. This study was supported by a grant from the Korea Healthcare Technology R&D Project, Ministry of Health and Welfare, Republic of Korea (A102065). Clinical Trial Registration Number: NCT00886574.

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