



Comparative Study of *Ex Vivo* Antiplatelet Activity of Aspirin and Cilostazol in Patients with Diabetes and High Risk of Cardiovascular Disease

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Background: The role of aspirin in primary cardiovascular disease prevention in patients with diabetes remains controversial. However, some studies have suggested beneficial effects of cilostazol on cardiovascular disease in patients with diabetes. We prospectively investigated the antiplatelet effects of cilostazol compared with aspirin in patients with diabetes and cardiovascular risk factors.

Methods: We randomly assigned 116 patients with type 2 diabetes and cardiovascular risk factors but no evident cardiovascular disease to receive aspirin at a dose of 100 mg or cilostazol at a dose of 200 mg daily for 14 days. The primary efficacy outcome was antiplatelet effects of aspirin and cilostazol assessed with the VerifyNow system (aspirin response units [ARU]) and PFA-100 (closure time [CT]). Secondary outcomes were changes of clinical laboratory data (ClinicalTrials.gov Identifier: NCT02933788).

Results: After 14 days, there was greater decrease in ARU in aspirin ($-28.9\% \pm 9.9\%$) compared cilostazol ($-0.4\% \pm 7.1\%$, $P < 0.001$) and was greater increase in CT in aspirin ($99.6\% \pm 63.5\%$) compared cilostazol ($25.7\% \pm 54.1\%$, $P < 0.001$). The prevalence of aspirin resistance was 7.5% according to VerifyNow (defined by $ARU \geq 550$) and 18.9% according to PFA-100 ($CT < 192$ seconds). Compared with aspirin, cilostazol treatment was associated with increased high density lipoprotein cholesterol ($7.1\% \pm 12.7\%$ vs. $4.2\% \pm 18.0\%$, $P = 0.006$) and decreased triglycerides ($-9.4\% \pm 33.7\%$ vs. $4.4\% \pm 17.57\%$, $P = 0.016$). However, there were no significant changes in total and low density lipoprotein cholesterol, C-reactive protein level, and cluster of differentiation 40 ligand between cilostazol and aspirin groups.

Conclusion: Aspirin showed better antiplatelet effects assessed with VerifyNow and PFA-100 compared with cilostazol. However, there were favorable changes in atherogenic dyslipidemia only in the cilostazol.

Keywords: Aspirin; Cardiovascular diseases; Cilostazol; Platelet aggregation; Platelet aggregation inhibitors

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INTRODUCTION

The balance of benefits and risks associated with usage of medication must be considered in various clinical situations. Aspirin has been shown to have an overall net clinical benefit (cardiovascular benefit vs. bleeding risk) when used for secondary prevention of atherosclerotic cardiovascular disease (ASCVD) in people with and without diabetes [1]. However, aspirin as secondary prevention reduced the risk of ASCVD in patients with diabetes by <10% compared with a >20% reduction in patients without diabetes [2]. Furthermore, several randomized studies showed that the benefit of aspirin did not overcome the risk in primary prevention of ASCVD among patients with type 2 diabetes mellitus [3,4].

The concept of “aspirin resistance” has been proposed to explain the poor response to aspirin to obtain adequate platelet inhibition in patients with type 2 diabetes mellitus [5,6]. Aspirin resistance is categorized as laboratory aspirin resistance, which is platelet reactivity not appropriately blocked by aspirin usage, and clinical aspirin resistance, which is failure of prevention of ASCVD events in patients taking aspirin [7]. Various testing methods have been developed to evaluate aspirin resistance. Light or optical transmission aggregometry has suggested that one in four diabetic patients taking aspirin had resistance [8]. If patients with diabetes do not respond to aspirin therapy, it may not be an adequate primary prevention therapy for patients with diabetes, and an alternative is needed.

Cilostazol, a reversible, selective inhibitor of phosphodiesterase 3, was shown to inhibit platelet activation in both *in vitro* and *in vivo* examinations [9]. Cilostazol is broadly used for treatment of ischemic stroke, transient ischemic attack, and peripheral arterial disease [10]. A previous open-label, single-arm, uncontrolled study showed that cilostazol significantly attenuated platelet activation, as measured using a laser light scattering aggregometer under no stimulation with exogenous agonists, in type 2 diabetes mellitus patients with insufficient platelet response to aspirin [11]. However, no randomized study has yet compared antiplatelet activity between aspirin and cilostazol.

We performed the ESCORT-DM (Effect of aspirin versus cilostazol for inhibition of antiplatelet aggregation in type 2 diabetes mellitus patients) randomized trial to compare antiplatelet efficacy, cardiovascular risk markers, and safety between aspirin and cilostazol in high risk Korean patients with type 2 diabetes mellitus.

METHODS

Study design and participants

This study was a randomized, open-label, active-controlled, parallel-group, multi-center study. Participants eligible for the study were patients aged 50 years or older with type 2 diabetes mellitus and one or more cardiovascular risk factors (family history of cardiovascular disease, hypertension, smoking history, dyslipidemia, and albuminuria) and without a high risk of bleeding. We excluded participants who were taking cilostazol or aspirin within one month before randomization. Other exclusion criteria included type 1 diabetes mellitus, secondary diabetes, or gestational diabetes; history of macrovascular complication including cardiovascular disease, cerebrovascular disease, and peripheral vascular disease; contraindicated for aspirin or cilostazol; clinically significant thyroid-stimulating hormone value outside the normal range; alanine aminotransferase or aspartate aminotransferase ≥ 2.5 times the upper limit of the normal range; alcohol intake greater than 30 g/day; presence of liver cirrhosis or tumor; continuous use (more than 2 weeks) of anti-thrombotic agents (sarpogrelate, beraprost, indobufen, triflusal, clopidogrel, and ticlopidine) or nonsteroidal anti-inflammatory drugs within 1 month or after randomization; current use of warfarin, dicoumarin derivatives, or digoxin; pregnant, nursing, or suspected of being pregnant; and history of gastrectomy. After screening, eligible and consenting participants underwent baseline evaluation (including anthropometric and lifestyle data, vital signs, medical history and concomitant medication, and venous blood and urine samples). All patients were then randomized into two groups: aspirin 100 mg every day or cilostazol 200 mg every day (1:1 matching). The randomization was based on the randomization table according to registration order. The study duration was determined as 14 days based on the previous study for aspirin resistance and with the consideration for the platelet life span (8 to 10 days) [12-14]. After a 14-day treatment period, participants visited the investigational site and underwent follow-up evaluation (vital signs and venous blood and urine samples); adverse reactions were reported, and the number of remaining pills was determined for evaluation of compliance.

The study was conducted at two university hospitals in Korea between October 2016 and July 2019 (ClinicalTrials.gov Identifier: NCT02933788). The study was conducted in accordance with the principles of Good Clinical Practice and was approved by the appropriate Institutional Review Boards of Kangbuk Samsung Hospital (2015-10-032) and Asan Medical Center

(2016-0649) for the study protocol. All patients provided written informed consent in accord with the Declaration of Helsinki, prior to participation.

Patient and public involvement

The patients or the public were not involved in the design, conduct, reporting, or dissemination plans of our research.

Study outcomes

The primary outcome measure of this study was change in platelet reactivity from baseline to day 14. Platelet reactivity was tested using the Platelet Function Analyzer 100 (PFA-100, Dade Behring, Miami, FL, USA) and the VerifyNow Aspirin instrument (Accumetrics Inc., San Diego, CA, USA). The secondary outcome measures were changes in lipid profiles such as total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides; C-reactive protein (CRP) level; and cluster of differentiation 40 ligand (CD40L) after 14 days of treatment. Safety was assessed by recording major bleeding events (intracranial, gastrointestinal, or other), adverse events (AEs), complete blood count, blood urea nitrogen-to-creatinine ratio, and aspartate aminotransferase/alanine aminotransferase. Adherence to the trial regimen was assessed by pill count, and 70% and lower adherence was defined as nonadherence.

Anthropometric and laboratory measurements

Questionnaires for diabetes duration; current and past medication history; and past medical history for cardiovascular disease, alcohol history, and smoking status were completed. Anthropometric measures (height, weight, waist circumference), systolic blood pressure, diastolic blood pressure, heart rate, and clinical laboratory data were assessed at baseline and after 14 days of treatment. Venous blood and urine samples were obtained in the morning after a 12-hour overnight fast and 2 hours after intake of trial regimen. Hemoglobin A1c level was measured using high performance liquid chromatography. Serum insulin level was measured using an immunoradiometric assay. Insulin resistance was estimated using homeostatic model assessment for insulin resistance (HOMA-IR), defined as $[\text{fasting plasma insulin (mU/L)} \times \text{fasting plasma glucose (mmol/L)}] / 22.5$ [15]. Beta-cell function was estimated using homeostatic model assessment for β cell function (HOMA- β), calculated as $\text{fasting plasma insulin } (\mu\text{U/mL}) \times 20 / \text{fasting plasma glucose (mmol/L)} - 3.5$ [15]. Chemistry values were determined using standard assays in each local laboratory. Glomerular filtration rate was estimated

by the Modification of Diet in Renal Disease Study Group formula [16]. The levels of CRP and CD40L were measured using a high sensitivity enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA).

PFA-100

Blood was aspirated under constant vacuum from the sample reservoir through a capillary and a microscopic hole (147 μm) in the membrane by PFA-100. Platelet activation evaluated by PFA-100 is based on co-stimulation of platelets by high shearing stress with a capillary and on the contact of platelets with a membrane coated with collagen and epinephrine to form a platelet plug within the hole. Platelet function was quantitated as the time necessary for thrombotic occlusion of a hole in a membrane coated with collagen and epinephrine (closure time [CT]) [17]. A CT of 193 seconds or less indicated normal platelet function, whereas a CT over 300 seconds was considered non-closure according to the manufacturer's cut-off values.

VerifyNow aspirin

The VerifyNow system evaluates platelet activity by measuring the light absorbance through the sample. VerifyNow contains a lyophilized preparation containing human fibrinogen-coated beads that cross-links with activated platelets. When platelets become activated by the specific agonist, the fibrinogen-coated beads agglutinate with platelets, and light transmission increases. The VerifyNow Aspirin test uses arachidonic acid as the specific agonist, which is converted by the cyclooxygenase-1 (COX-1) enzyme (the molecular target of aspirin therapy) into thromboxane A₂. The data are output as aspirin response units (ARUs), and a cut-off value of $\text{ARU} \geq 550$ was accepted to exclude aspirin-induced platelet aggregation according to the manufacturer's reference values.

Statistical analysis

The sample size was calculated based on previous studies. The change of platelet aggregation rate (D) between baseline and after administration of aspirin or cilostazol was defined as follows:

$$\text{change of platelet aggregation rate} = \frac{(\text{baseline rate}) - (\text{after administration rate})}{(\text{baseline rate})} \times 100$$

According to a previous study, the change of platelet aggregation rate with arachidonic acid after aspirin and cilostazol administration were 0.4504 ± 0.3651 and 0.69 ± 0.4975 , respectively [18]. A sample size of 58 patients per trial group was esti-

mated to provide the trial with 80% power (2-sided, 5% significance) while considering 10% dropout of study participants.

Categorical variables are expressed as frequency and percentage. Continuous variables are presented as mean±standard deviation. Continuous variables were analyzed for a normal distribution with the Shapiro-Wilks goodness-of-fit test (using $P<0.1$ as threshold). Only patients with drug adherence greater than 70% were considered for comparisons. Paired t tests were used for comparison of normally distributed continuous variables in the same group. Wilcoxon tests were used for paired comparisons of continuous variables not following a normal distribution. The primary and secondary outcomes between the two groups were compared using repeated measures analysis of variance (ANOVA). Safety comparisons were assessed using chi-square for proportions. All statistical analyses were performed using IBM SPSS software version 24.0 (IBM Co., Armonk, NY, USA). Two-tailed values of $P<0.05$ were considered statistically significant.

RESULTS

A total of 127 subjects was screened, and 116 eligible subjects were randomly assigned to the aspirin group ($n=58$) or cilostazol group ($n=58$). Two subjects in the aspirin group withdrew consent before the baseline test. A total of 114 subjects (56 in aspirin group, 58 in cilostazol group; full analysis set) performed the baseline test. Demographic information and baseline characteristics of the full analysis set subjects enrolled in this study are summarized in Table 1. The mean age of the study subjects was 60.0 ± 6.6 years, and 76 subjects (66.7%) were men. There were no significant differences in baseline characteristics between groups (Table 1).

In the aspirin group, two patients were excluded due to side effects (abdominal pain=1, thrombocytopenia=1) and one patient due to low compliance. In the cilostazol group, 11 patients were excluded due to side effects (headache=9, dizziness=1, palpitations=1) and 10 patients due to low compliance. A final 90 subjects (53 in the aspirin group, 37 in the cilostazol group; per protocol analysis set) completed the study (Supplemental Fig. S1). We analyzed the primary and secondary outcomes of the per protocol analysis set (Supplemental Table S1).

Effects of aspirin and cilostazol on platelet reactivity

The platelet reactivity change after 14 days of cilostazol or aspirin treatment was determined by changes in VerifyNow (ARU) and PFA-100 (CT) values (Table 2). In the cilostazol group,

Table 1. Baseline Characteristics of Study Participants According to Treatment Group (Full Analysis Set)

Characteristic	Cilostazol	Aspirin	<i>P</i> value
Number	58	56	
Age, yr	59.8±6.8	60.1±6.3	0.810
Male sex	43 (74.1)	33 (58.9)	0.112
Height, cm	166±8	164±7	0.162
Weight, kg	69±9	67±12	0.243
Smoking	15 (25.9)	7 (12.5)	0.097
Non-drinkers	41 (70.7)	41 (73.2)	0.836
Waist circumference, cm	90±8	88±10	0.212
Systolic blood pressure, mm Hg	126±14	128±16	0.411
Diastolic blood pressure, mm Hg	76±11	76±11	0.704
Heart rate, bpm	75±11	78±11	0.183
Previous cilostazol exposure	7 (12.1)	6 (10.7)	1.000
History of cardiovascular disease	4 (6.9)	4 (7.1)	1.000
Hypertension	28 (48.3)	29 (51.8)	0.852
Dyslipidemia	51 (87.9)	49 (87.5)	1.000
Albuminuria	0	0	
White blood cells, $\times 10^3/\text{mm}^3$	6.13±1.47	6.55±1.57	0.143
Hemoglobin, g/dL	14.4±1.5	13.9±1.5	0.063
Platelet count, $\times 10^3/\mu\text{L}$	226±63	229±60	0.752
Fasting glucose, mg/dL	132±29	146±42	0.040
HbA1c, %	7.0±1.3	7.3±1.3	0.199
Fasting insulin, $\mu\text{IU}/\text{mL}$	8.53±3.86	9.76±9.51	0.375
HOMA-IR	2.82±1.49	3.55±3.42	0.145
HOMA- β , %	50.3±25.4	53.0±68.3	0.782
Total cholesterol, mg/dL	140±41	140±33	0.970
Triglycerides, mg/dL	120±65	151±77	0.024
HDL-cholesterol, mg/dL	52±11	52±19	0.980
LDL-cholesterol, mg/dL	83±38	79±30	0.457
Blood urea nitrogen, mg/dL	14.9±3.5	16.1±5.4	0.160
Serum creatinine, mg/dL	1.43±4.75	1.49±4.97	0.947
eGFR, mL/min/1.73 m ²	92.7±10.9	88.8±16.8	0.146
AST, IU/L	24±8	25±12	0.519
ALT, IU/L	25±13	24±14	0.946
VerifyNow, ARU	623±61	637±40	0.696
PFA-100, sec	137±41	140±50	0.754
CRP, ng/mL	1,271±4,319	868±1,368	0.501
CD40 ligand, pg/mL	1,880±1,470	1,703±1,624	0.541
Diabetes medication			
Metformin	39 (67.2)	44 (78.6)	0.209
Sulfonylurea	13 (22.4)	23 (41.1)	0.044
Thiazolidinedione	4 (6.9)	5 (8.9)	0.740
DPP-4 inhibitor	32 (55.2)	37 (66.1)	0.256
SGLT2 inhibitor	13 (22.4)	10 (17.9)	0.643

(Continued to the next page)

Table 1. Continued

Characteristic	Cilostazol	Aspirin	P value
Hypertension medication	25 (43.1)	24 (42.9)	1.000
Angiotensin II receptor blocker	20 (34.5)	14 (25.0)	0.310
ACE inhibitor	1 (1.7)	2 (3.6)	0.615
Diuretics	0	1 (1.8)	0.491
Calcium channel blockers	6 (10.3)	7 (12.5)	0.775
β-Blocker	2 (3.4)	2 (3.6)	1.000
Other hypertension medication	3 (5.2)	4 (7.1)	0.741
Dyslipidemia medication	53 (91.4)	53 (94.6)	0.717
Statin	51 (87.9)	53 (94.6)	0.322
Fibrate	2 (3.4)	2 (3.6)	1.000
Other dyslipidemia medication	6 (10.3)	6 (10.7)	1.000

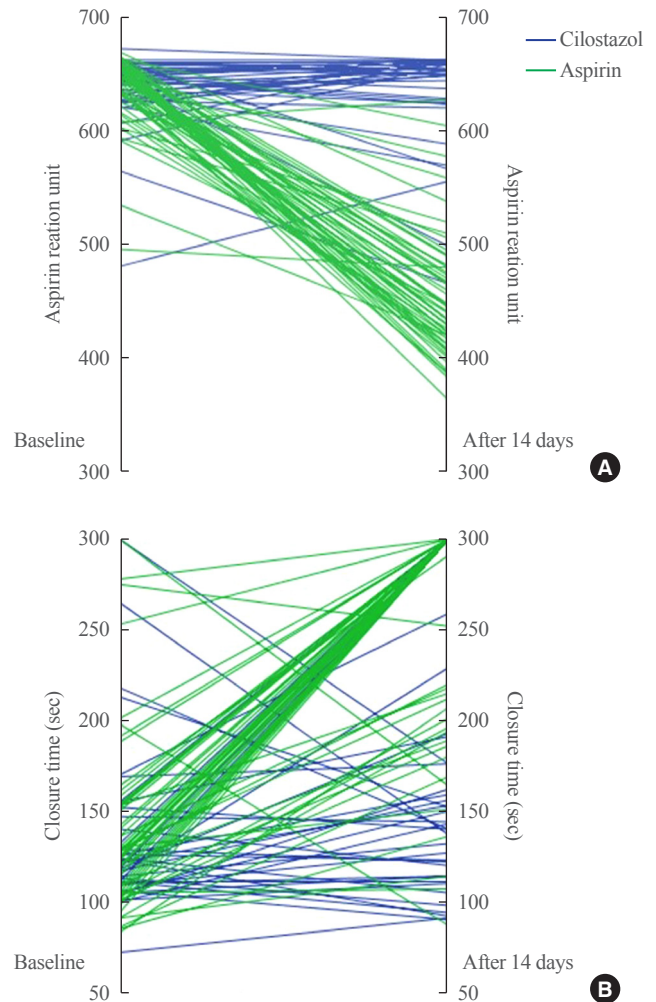
Values are expressed as mean ± standard deviation or number (%). HbA1c, hemoglobin A1c; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment for β cell function; HDL, high density lipoprotein; LDL, low density lipoprotein; eGFR, estimated glomerular filtration rate; AST, aspartate transferase; ALT, alanine transaminase; ARU, aspirin response units; CRP, C-reactive protein; CD40, cluster of differentiation 40; DPP-4, dipeptidyl peptidase 4; SGLT-2, sodium-glucose cotransporter-2; ACE, angiotensin-converting-enzyme.

Table 2. VerifyNow and PFA-100 Value Changes after 14 Days of Cilostazol or Aspirin Treatment

Variable	Cilostazol group	Aspirin group	P value
VerifyNow, ARU			
Baseline	637 ± 34	639 ± 32	0.759
14 days	634 ± 46	453 ± 56	<0.001
Changes over 14 days, %	-0.4 ± 7.1	-28.9 ± 9.9 ^a	<0.001
PFA-100, CT			
Baseline	136 ± 46	138 ± 32	0.832
14 days	162 ± 66	256 ± 63	<0.001
Changes over 14 days, %	25.7 ± 54.1 ^b	99.6 ± 63.5 ^b	<0.001

Values are expressed as mean ± standard deviation. ARU, aspirin resistance units; CT, closure time. ^a $P < 0.05$; ^b $P < 0.001$.

there was no significant ARU change after the 14-day treatment (mean change: $-0.4\% \pm 7.1\%$, $P = 0.632$), but there was significant increase in CT (mean change: $25.7\% \pm 54.1\%$, $P = 0.043$) (Fig. 1). In the aspirin group, there was a significant decrease in ARU after the 14-day treatment (mean change: $-28.9\% \pm 9.9\%$, $P < 0.001$) and an increase in CT (mean change: $99.6\% \pm 63.5\%$,

**Fig. 1.** (A) VerifyNow and (B) PFA-100 value changes after 14 days of cilostazol or aspirin treatment.

$P < 0.001$). Compared with the results in the cilostazol group, there was significant decrease in ARU ($P < 0.001$) and increase in CT ($P < 0.001$) after 14-day treatment in the aspirin group (Table 2). Aspirin resistance was defined as ARU ≥ 550 or CT < 192 seconds, and the prevalence of aspirin resistance was 7.5% according to VerifyNow and 18.9% according to PFA-100 (Fig. 2).

Effects of aspirin and cilostazol on lipid profile, CRP, and CD40L

After 14 days of treatment, in the cilostazol group, there were significant changes in triglycerides (mean change: $-9.4\% \pm 33.7\%$, $P = 0.019$) and HDL-cholesterol levels (mean change: $7.1\% \pm 12.7\%$, $P = 0.001$) but no significant change in total cholesterol (mean change: $0.71\% \pm 17.22\%$, $P = 0.979$) or LDL-

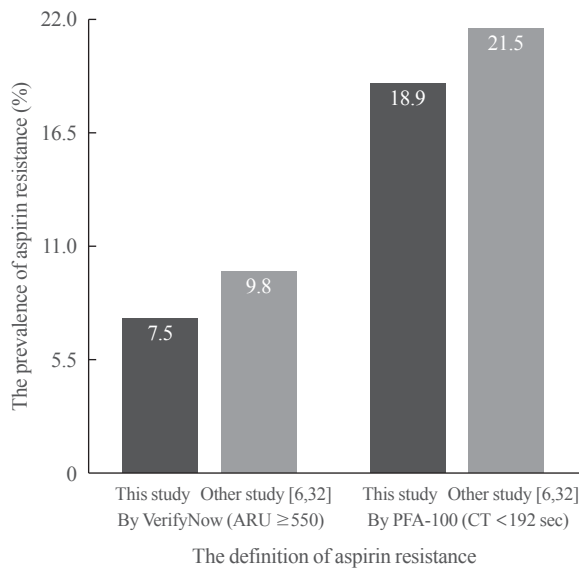


Fig. 2. The prevalence of aspirin resistance. ARU, aspirin resistance units; CT, closure time.

cholesterol (mean change: $0.89\% \pm 27.60\%$, $P=0.946$) (Table 3). In the aspirin group, there was no significant change in total cholesterol (mean change: $1.93\% \pm 17.57\%$, $P=0.902$), triglycerides (mean change: $4.4\% \pm 17.57\%$, $P=0.953$), HDL-cholesterol (mean change: $4.2\% \pm 18.0\%$, $P=0.480$), or LDL-cholesterol (mean change: $7.19\% \pm 32.09\%$, $P=0.399$). Compared with results in the aspirin group, there were significant improvements of triglyceride ($P=0.016$) and HDL-cholesterol levels ($P=0.006$) in the cilostazol group but no difference in total cholesterol ($P=0.956$) and LDL-cholesterol levels ($P=0.696$) (Table 3). After the 14-day treatment, there was no significant change in CRP or CD40 ligand in either group compared with baseline and no significant difference in changes of CRP and CD40 ligand between the two groups (Table 3).

Safety and AEs

Safety and AEs are listed in Table 4. In both study groups, there were no serious AEs. However, the numbers of AEs leading to discontinuation of the trial regimen were higher in the cilostazol group ($n=11$) than in the aspirin group ($n=2$, $P=0.01$). The proportion of subjects with poor adherence was higher in the cilostazol group ($n=10$) than in the aspirin group ($n=1$, $P=0.005$). Headache was more common in the cilostazol group (27.6%) than in the aspirin group (1.8%: hazard ratio [HR], 20.1; 95% confidence interval [CI], 2.7 to 164.4; $P=0.004$). Abdominal discomfort was similar in the cilostazol and aspirin groups (3.4% vs. 1.8%: HR, 1.47; 95% CI, 0.30 to 7.45; $P=0.579$). In

Table 3. Lipid Profile, CRP, and CD40L Level Changes after 14 Days of Cilostazol or Aspirin Treatment

Variable	Cilostazol group	Aspirin group	P value
Total cholesterol, mg/dL			
Baseline	134±46	139±32	0.480
14 days	134±33	138±32	0.576
Changes over 14 days, %	0.71±17.22	1.93±17.57	0.956
Triglycerides, mg/dL			
Baseline	116±68	147±75	0.044
14 days	95±49	146±96	0.005
Changes over 14 days, %	-9.4±33.7 ^a	4.4±49.2	0.016
HDL-cholesterol, mg/dL			
Baseline	54±12	52±20	0.607
14 days	57±13	53±17	0.158
Changes over 14 days, %	7.1±12.7 ^b	4.2±18.0	0.006
LDL-cholesterol, mg/dL			
Baseline	76±27	77±29	0.834
14 days	76±33	79±29	0.634
Changes over 14 days, %	0.89±27.60	7.19±32.09	0.696
CRP, ng/mL			
Baseline	1,706±5,355	902±1,417	0.299
14 days	952±1,124	1,285±3,876	0.561
Changes over 14 days, %	68±162	175±1,087	0.681
CD40 ligand, pg/mL			
Baseline	1,992±1,432	1,747±1,621	0.451
14 days	2,309±1,593	1,609±1,524	0.042
Changes over 14 days, %	153.7±669.4	85.0±232.2	0.674

Values are expressed as mean±standard deviation.

CRP, C-reactive protein; CD40, cluster of differentiation 40; HDL, high density lipoprotein; LDL, low density lipoprotein.

^a $P<0.05$; ^b $P<0.01$.

the cilostazol group, common cold (1.7%), myalgia (3.4%), epistaxis (1.7%), palpitation (1.7%), and dizziness (1.7%) were reported. Decrease in platelet count (1.7%) caused discontinuation of the trial regimen in the aspirin group.

DISCUSSION

This 14-day randomized trial revealed better *ex vivo* antiplatelet effects of aspirin as assessed with VerifyNow and PFA-100 compared with cilostazol in patients with type 2 diabetes mellitus. Regarding secondary outcomes, cilostazol significantly improved atherogenic dyslipidemia, with increased HDL-choles-

Table 4. Adverse Events

Variable	Cilostazol	Aspirin	P value
Serious adverse events	0	0	NS
Adverse event leading to discontinuation of trial regimen	11 (19.0)	2 (3.6)	0.01
Poor adherence to trial regimen	10	1	0.005
Headache	16 (27.6)	1 (1.8)	0.004
Abdominal discomfort	2 (3.4)	1 (1.8)	0.579
Decrease PLT count	0	1 (1.8)	NS
Common cold	1 (1.7)	0	NS
Myalgia	2 (3.4)	0	NS
Epistaxis	1 (1.7)	0	NS
Palpitation	1 (1.7)	0	NS
Dizziness	1 (1.7)	0	NS

Values are expressed as number (%).
PLT, platelet; NS, not significant.

terol level and decreased triglyceride level compared with aspirin treatment. Regarding AEs and adherence, aspirin showed better tolerability than cilostazol.

Aspirin and cilostazol are all antiplatelet drug, but pharmacodynamics of them are different on platelet function. Aspirin produces the antiplatelet effect through the irreversible inhibition of thromboxane and cilostazol produces the antiplatelet effect through increasing the level of cyclic adenosine monophosphate by inhibiting phosphodiesterase 3. Few studies have compared the antiplatelet activity between aspirin and cilostazol. *In vitro* study, the effects of 100 $\mu\text{mol/L}$ aspirin and 10 $\mu\text{mol/L}$ cilostazol were similar in inhibiting platelet aggregation [19]. Only one randomized crossover study compared the *ex vivo* antiplatelet efficacy between aspirin and cilostazol [18]. This comparative study in 12 healthy men showed that cilostazol was as effective as aspirin and clopidogrel in inhibiting *ex vivo* platelet aggregation, induced by the aggregation inducers adenosine diphosphate, collagen, epinephrine, and arachidonic acid, without prolonging bleeding time or changing the bleeding pattern compared with aspirin and clopidogrel [18]. Platelet aggregation activity was measured in platelet-rich plasma at 37°C using an aggregometer (CHRONO, 490 2D). However, in our study with diabetes patients, the antiplatelet efficacy of aspirin was superior to that of cilostazol as assessed with the VerifyNow Aspirin system ($P < 0.001$) and PFA-100 ($P < 0.001$). The discrepancy between study results may be explained by the methodological differences in platelet function testing and the difference in populations (subjects with vs. without diabetes, smoking status, or

medications such as statin).

There are several platelet function tests used in clinical studies to assess the reactivity of platelets, each with their own benefits and limitations. Point-of-care devices, such as the VerifyNow Aspirin system, had benefits with operating at a patient's bedside, and with minimal expertise. VerifyNow Aspirin system which using arachidonic acid as the specific agonist assess thromboxane or COX-1 dependent pathways which is closely related to aspirin pharmacodynamics. PFA-100 assesses platelet function in an alternative method through a non-COX-1-dependent method which mimics the *in vivo* environment using a whole blood sample with high shear force [20]. Our study results indicated that cilostazol failed to show platelet aggregation inhibition as assessed with the VerifyNow Aspirin system (mean change: $-0.4\% \pm 7.1\%$; $P = 0.632$) but showed significant inhibition as assessed with PFA-100 (mean change: $25.7\% \pm 54.1\%$; $P = 0.043$). These findings might be because the VerifyNow Aspirin system is not adequate to assess the antiplatelet activity of cilostazol. Cilostazol act upstream of COX-1 pathway by suppressing the release of arachidonic acid in platelets, therefore direct arachidonic acid stimulation of the VerifyNow Aspirin system may bypass the action of cilostazol [21,22]. Several previous studies also failed to identify antiplatelet activity of cilostazol with the VerifyNow Aspirin system. In *ex vivo* studies, after a single oral uptake of cilostazol, the VerifyNow IIb/IIIa test and the VerifyNow P2Y12 test detected a positive inhibitory effect of cilostazol, but the VerifyNow Aspirin test was not able to detect these results [23-25]. Although cilostazol showed significant inhibition of platelet aggregation as assessed with PFA-100 (mean change: $25.7\% \pm 54.1\%$, $P = 0.043$), it was inferior to aspirin (mean change: $99.6\% \pm 63.5\%$ vs. $25.7\% \pm 54.1\%$, $P < 0.001$). In a previous animal model study, cilostazol showed anti-thrombotic effects *in vivo* at much lower plasma concentrations than the effective concentrations measured in *ex vivo* or *in vitro* aggregation tests using PFA-100 [26]. And recent study reported showed that the *ex vivo* inhibitory effect of cilostazol on platelet was clearly detected with the present of prostaglandin E1 (PGE1) [23,27]. And it supported by other study which showed that the addition of low concentrations of PGE1 results in an increase cyclic adenosine monophosphate of platelet which serves to amplify the inhibitory effect of cilostazol [28]. Therefore, there is some possibility that PFA-100 as an *ex vivo* test underestimates the antiplatelet activity of cilostazol.

Patients with diabetes are typically characterized by increased platelet reactivity and an increased level and activity of prothrombotic clotting factors [29]. The activation of platelets is a

complex process involving a number of steps which are not only COX-1-dependent steps, but also non-COX-1-dependent steps [30]. These may explain the predisposition to inadequate aspirin-induced effects named as “aspirin resistance.” And the discrepancy in cilostazol-induced effect between previous study (with health participants) and this study (with diabetes patients) could be explained by increased platelet reactivity in patients with diabetes, but further studies are needed. Smoking status or medications such as statin could attenuate the antiplatelet efficacy of aspirin and cilostazol, there were not significant difference between aspirin group and cilostazol group (Table 1).

The prevalence of aspirin resistance in our study was 7.5% according to VerifyNow and 18.9% according to PFA-100, and these results are similar to a previous study (Fig. 2). According to our previous study of 1,056 type 2 diabetes mellitus patients from 11 hospitals, aspirin resistance measured in ARUs using VerifyNow was detected in 102 of 1,045 subjects (prevalence 9.8%) and was associated with HDL-cholesterol [6]. Another study of patients with type 2 diabetes mellitus reported that the prevalence of aspirin resistance measured by the PFA-100 system was 21.5% [31]. These findings are consistent with our study results. In our study, the prevalence of aspirin resistance according to PFA-100 was higher than that according to VerifyNow. It could be explained that PFA-100 measures a platelet reactivity caused by shear stress, collagen, and epinephrine stimulation, which cannot be expected to be completely inhibited by aspirin, and many variables, which can affect the results of PFA-100 but not VerifyNow, including platelet count, red blood cells, platelet reactivity to collagen, and plasma von Willibrand factor [32,33].

Unlike the previously mentioned platelet reactivity results, cilostazol treatment for only 14 days improved triglyceride ($P=0.016$) and HDL-cholesterol ($P=0.006$) levels comparing with aspirin treatment in our study. Although the mechanism for the beneficial effects on dyslipidemia by cilostazol is not still clear [34], these results are consistent with our previous randomized, open, 36-month, multi-center trial that also showed improved triglyceride and HDL-cholesterol levels in the cilostazol treatment group compared with the aspirin treatment group [35], and other randomized studies showed similar results [36]. However, CRP and CD40 ligand were unchanged in both groups in this study. Some studies reported that aspirin and cilostazol reduced CRP or CD40 ligand levels, but other studies reported no changes [37-39]. These discrepancies could be due to several factors. These studies used heterogeneous and diverse populations, including healthy subjects, patients with diabetes,

and patients with ischemic heart disease, and each study reported different baseline CRP and CD40 ligand levels. In addition, the duration of aspirin or cilostazol treatment varied among studies. In our study, the 14-day treatment period might be too short to detect changes of CRP and CD40 ligand levels by aspirin or cilostazol treatment.

Regarding safety and AEs, the aspirin group showed a lower rate of AEs and higher adherence to medication. Headache was most common side effect in the cilostazol group and caused many withdrawals from the study in this group (15.5%; 9/58 patients). This withdrawal rate was similar to that of a previous study (16%) [40]. Although previous studies showed that headache due to cilostazol resolved after several weeks, our study was too short to observe this finding [40]. Headache was associated with low adherence to cilostazol in our studies. As this low adherence could result in low efficacy of cilostazol compared with aspirin, we excluded patients with low adherence to trial regimens.

To our knowledge, this is the first randomized study comparing the *ex vivo* antiplatelet efficacy, evaluated by VerifyNow and PFA-100, and the safety of aspirin and cilostazol in type 2 diabetes mellitus patients. The strength of this study is its prospective and randomized design using the most popular point-of-care tests (VerifyNow and PFA-100). This study also has some limitations. First, as the mechanisms of aspirin and cilostazol are different, some platelet function tests were appropriate with some medication but inappropriate with others. To overcome this problem, we used two popular platelet function tests based on platelet aggregation (VerifyNow) or platelet adhesion under shear stress (PFA-100) [17]. Second, the short-term study period (14 days) can result in bias in evaluating safety. This short duration may not be sufficient for development of major gastrointestinal bleeding, which is a common AE of aspirin [18], although it was adequate to evaluate the primary outcome.

In summary, aspirin as assessed by VerifyNow and PFA-100 showed higher efficacy and tolerability compared with cilostazol. However, the favorable changes in atherogenic dyslipidemia (triglycerides and HDL-cholesterol) only in the cilostazol treatment group indicate additional benefits with long-term administration of cilostazol. We also anticipate synergistic effects of the combination of aspirin and cilostazol on preventing ASCVD. A future study with long duration and another methodology to assess the antiplatelet efficacy of cilostazol and aspirin is needed.

CONFLICTS OF INTEREST

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AUTHOR CONTRIBUTIONS

Conception or design: W.J.L., C.Y.P. Acquisition, analysis, or interpretation of data: S.H., W.J.L., C.Y.P. Drafting the work or revising: S.H. Final approval of the manuscript: W.J.L., C.Y.P.

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