Platelets play a central role in the pathogenesis of atherothrombosis, so platelet inhibition is an important part of managing a patient with an atherothrombotic event. Dual antiplatelet therapy using clopidogrel plus aspirin reduces ischemic events in patients with unstable angina and myocardial infarction (MI), especially those undergoing percutaneous coronary intervention (PCI) and stenting, but despite this proven benefit, there is considerable heterogeneity in the responses of individual patients to aspirin and to clopidogrel. Emerging data show adequate antiplatelet effects are not achieved in 5–45% of patients taking aspirin and in 4–30% of patients taking clopidogrel, suggesting that many patients are resistant or only partially responsive to the antiplatelet effect. Some data suggest that these patients are at increased risk of stent thrombosis and cardiovascular complications.

Cilostazol is a potent, oral antiplatelet agent with a rapid onset of action that selectively inhibits phosphodiesterase 3 and increases cyclic adenosine monophosphate (cAMP) levels in platelets. The increase in cAMP blocks all activating pathways in the platelets, inhibiting processes such as adenosine diphosphate (ADP) induced platelet aggregation, arachidonic acid (AA) induced platelet activation, and cellular interactions among platelets, leukocytes, and vascular endothelial cells.

Recent studies have shown that adding cilostazol to the combination of aspirin plus clopidogrel significantly increases the inhibition of ADP-induced platelet aggregation. However, there was no additive effect on aspirin-induced antiplatelet activity or lowering of sCD40L.

**Methods**

**Study Population**

We enrolled 60 consecutive patients with ST elevation MI (STEMI) undergoing primary PCI within 12 h of symptom onset. The patients were randomly assigned 1:1 to receive either a standard dual regimen (aspirin and clopidogrel) or a triple regimen (aspirin, clopidogrel, and cilostazol). Ex-
Eligible patients (n=83)  
Exclusions (n=17)  
Refused (n=6)  
Randomization (n=60)  
Dual regimen (n=30)  
: Aspirin (A) + clopidogrel (C)  
Loading dose  
A: 300 mg and C: 600 mg  
Primary PCIs with drug eluting stent implantation  
Maintain daily dose  
A: 100 mg and C: 75mg  
VeryfYN-now-aspirin and -P2Y12 assay during 7-21 days (n=30)  
Triple regimen (n=30)  
: A + C + cilostazol (Ci)  
Loading dose  
A: 300 mg, C: 600 mg and Ci: 400 mg  
Maintain daily dose  
A: 100 mg, C: 75 mg and Ci: 200 mg  
First blood sample for sCD40L at random  
Second blood sample for sCD40L at 24 h after random  
Third blood sample for sCD40L at 21 d after random  

Table 1 Clinical Characteristics of the Study Subjects

<table>
<thead>
<tr>
<th></th>
<th>Dual (n=30)</th>
<th>Triple (n=30)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63.9±9.0</td>
<td>62.3±11.1</td>
<td>0.571</td>
</tr>
<tr>
<td>Male (%)</td>
<td>21 (70)</td>
<td>21 (70)</td>
<td>1.000</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>167.1±47.8</td>
<td>181.8±47.2</td>
<td>0.237</td>
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<tr>
<td>Hypertension (%)</td>
<td>14 (47)</td>
<td>17 (59)</td>
<td>0.571</td>
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<tr>
<td>Diabetes (%)</td>
<td>8 (27)</td>
<td>9 (31)</td>
<td>0.633</td>
</tr>
<tr>
<td>Hypercholesterolemia (%)</td>
<td>4 (13)</td>
<td>6 (20)</td>
<td>0.406</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>18 (60)</td>
<td>18 (60)</td>
<td>0.221</td>
</tr>
<tr>
<td>β-blocker (%)</td>
<td>19 (63)</td>
<td>23 (77)</td>
<td>0.266</td>
</tr>
<tr>
<td>ACEI (%)</td>
<td>10 (33)</td>
<td>12 (40)</td>
<td>0.691</td>
</tr>
<tr>
<td>ARB (%)</td>
<td>8 (27)</td>
<td>11 (37)</td>
<td>0.485</td>
</tr>
<tr>
<td>Statin (%)</td>
<td>22 (73)</td>
<td>20 (67)</td>
<td>0.485</td>
</tr>
<tr>
<td>Diuretics (%)</td>
<td>10 (33)</td>
<td>13 (43)</td>
<td>0.493</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>46.3±8.1</td>
<td>46.2±12.6</td>
<td>0.702</td>
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<td>Killip class, %</td>
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<td>0.215</td>
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<tr>
<td>I/II/III</td>
<td>80.8/11.5/7.7</td>
<td>92.0/0/0.0/8.0</td>
<td>0.166</td>
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<tr>
<td>Access site, %</td>
<td>90.0/10.0</td>
<td>76.7/23.3</td>
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<tr>
<td>Radial artery/femoral artery</td>
<td>15.4/53.8/30.8</td>
<td>12.0/40.0/48.0</td>
<td>0.648</td>
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<tr>
<td>Lesion type (A/B/C),%</td>
<td>79.2/16.7/4.2</td>
<td>84.0/8.0/8.0</td>
<td>0.290</td>
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<tr>
<td>Infarct-related artery, %</td>
<td>53.3/20.0/26.7</td>
<td>41.4/10.3/48.3</td>
<td>0.203</td>
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<tr>
<td>LAD/LCX/RCA</td>
<td>3.3%</td>
<td>6.7%</td>
<td>0.554</td>
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<tr>
<td>1 month MACE</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; LVEF, left ventricular ejection fraction; SES, sirolimus-eluting stent; PES, paclitaxel-eluting stent; LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; MACE, major adverse cardiac event.

Inclusion criteria were any contraindications for antiplatelet agents, severe left ventricular dysfunction (ejection fraction ≤30%), severe hepatic dysfunction (AST/ALT ≥3-fold upper normal limit), severe renal dysfunction (serum Cr ≥2), thrombocytopenia (<150×10^9/L), cardiogenic shock, infectious or neoplastic disease, and bleeding disorders (Fig 1). This study was approved by the Investigational Review Board of the Wonju College of Medicine. All patients gave written informed consent.

Study Protocol
As soon as the diagnosis of STEMI was confirmed in the
emergency department, we administered a loading dose of the antiplatelet regimens and a bolus of unfractionated heparin (70 U/kg) or enoxaparin (30 mg) to the patients between 20 and 50 min before primary PCI. The standard dual regimen group received 600 mg clopidogrel and 300 mg oral aspirin in the emergency room, followed by 75 mg clopidogrel and 100 mg aspirin daily thereafter. In the triple regimen group, patients received 600 mg clopidogrel, 300 mg oral aspirin and 400 mg cilostazol as loading dosages, followed by 75 mg clopidogrel, 100 mg aspirin, and 200 mg cilostazol daily for 1 month.

After coronary angiography via a transradial or transfemoral technique, primary PCI was performed using a standard technique for the infarct-related artery. Procedural success was defined as a residual diameter stenosis of <30% with Thrombolysis In Myocardial Infarction grade 3 flow. Major adverse cardiac events (MACE) were defined as death, recurrent MI and target vessel revascularization within 1 month. Major bleeding was defined as a decline in hemoglobin of ≥5 g/dl, significant hypotension requiring inotropes or surgery (other than vascular site repair), symptomatic intracranial hemorrhage, or requiring transfusion of 4 or more units of red blood cells.

VerifyNow-Aspirin/P2Y12 Assay and Measurement of sCD40L

Blood samples for sCD40L were obtained at baseline, 24h and 21 days from all participants. Concentrations of sCD40L in plasma were measured in duplicate with a standard enzyme-linked immunosorbent assay and a commercial kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The samples for in vitro platelet function were obtained after the 7th to 21st consecutive day of the study scheme when aspirin and clopidogrel were being administered at dosages of 100 mg daily and 75 mg daily respectively. All specimens were obtained by venipuncture and a 5-ml whole blood sample was drawn into 2 blood collection tubes (1.8-ml) containing 3.2% citrate and then analyzed independently.

In vitro platelet function testing for aspirin and clopidogrel was performed with the VerifyNow System (Accumetrics, San Diego, CA, USA), which is similar to the principle of light transmission aggregometry. The VerifyNow-aspirin system measures the change in the optical signal caused by aggregation, using cartridges containing fibrinogen-coated beads and platelet agonists (cationic propyl gallate which activates the cyclooxygenase-1 pathway). The results are expressed in Aspirin Reaction Units (ARUs), based on the extent of platelet aggregation. An ARU of ≥550 indicates the absence of aspirin-induced platelet dysfunction and is defined as aspirin resistance.17,18

The VerifyNow-P2Y12 system measures the change in the optical signal caused by aggregation, using cartridges containing fibrinogen coated beads and the platelet agonist ADP. Prostaglandin E1 is added to reduce the nonspecific contribution of the P2Y1 receptor. The results are expressed in P2Y12 reaction units (PRUs), based on the extent of platelet aggregation. An ARU of ≥550 indicates the absence of aspirin-induced platelet dysfunction and is defined as aspirin resistance.17,18

Inhibition. A lower responder to clopidogrel was defined as those patients with % inhibition of ADP-induced platelet aggregation <20.

Statistical Analysis

Continuous variables (presented as mean±SD) were compared by Student’s t-test for normally distributed variables and by the Wilcoxon test for non-normally distributed variables. Categorical variables were expressed as the number of subjects and percentages and were analyzed by the chi-square or Fisher’s exact test, as appropriate. Serial measurements of sCD40L between 2 groups were compared using repeated measures of AVOVA, and Scheffe’s multiple comparison tests was used to compare the mean different intervals. Multivariate stepwise logistic regression analyses...
were performed to identify independent predictors for low responders to clopidogrel as the dichotomous dependent variable. Statistical analysis was performed with the SPSS software package for Windows 12.0 (SPSS Inc, Chicago, IL, USA). P-values <0.05 were considered statistically significant.

Results

Baseline Characteristics and Clinical Outcomes

Baseline clinical, angiographic, and procedural characteristics were similar between the dual and triple regimens (Table 1). Procedural success was achieved in 100% in both groups. A complete 30-day follow-up was available for all eligible patients. MACE was 3.3% in the dual regimen group (1 case of recurrent non-Q wave MI) and 6.7% in the triple regimen group (2 cases of recurrent non-Q wave MI) (p=0.554). There was no major bleeding in either group, and no discontinuation of cilostazol because of adverse drug reactions.

In Vitro Platelet Function Test by VerifyNow for Aspirin and Clopidogrel Response

The mean ARUs for the dual and triple regimens were similar (dual 421.1±49.6 vs triple 426.4±62.1, p=0.717). The aspirin resistance rate (ARU ≥550) was also identical in both groups (3.4 vs 3.6%, p=0.960). However, the VerifyNow P2Y12 assay showed a significantly lower degree of PRU in the triple regimen group (168.2±79.2) than in the dual regimen group (208.8±69.0, p=0.041). The % inhibition of ADP-induced platelet aggregation was significantly higher in the triple regimen group than in the dual regimen group (dual 23.8±21.4 vs triple 40.5±21.1%, p=0.004; Fig 2). The rate of low responders to clopidogrel was significantly less in the triple regimen group than in the dual regimen group (dual 46.4 vs triple 15.4%, p=0.014).

In the multivariate logistic regression analysis using age, sex, risk factors and medications as independent variables and low responder to clopidogrel as a dependent variable, additional cilostazol (triple regimen) was the only independent negative risk factor for a lower responder to clopidogrel (odds ratio=0.219, 95% confidence interval 0.067–0.711; Table 2).

Serial Changes in sCD40L

The mean plasma sCD40L concentration at admission did not differ between the dual and triple regimen groups (dual 395.8±225.2 vs triple 346.6±489.5 pg/ml, p=NS). The level of sCD40L in plasma decreased in both groups at 24h compared with baseline, but the Δ change of sCD40L was not statistically significant between groups (dual 131.8±219.1 vs triple 110.9±186.5 pg/ml, p=NS). The plasma sCD40L concentration further decreased in both groups at 21 days compared with 24h, but the Δ change of sCD40L was also insignificant between both groups (dual 166.9±150.1 vs triple 148.0±157.6 pg/ml, p=NS; Fig 3).

Discussion

The main finding of the present study is that adding cilostazol to an aspirin and clopidogrel regimen significantly increases the inhibition of ADP-induced platelet aggregation compared with aspirin plus clopidogrel regimen alone in patients undergoing primary PCI. However, there was no additive or synergistic effect on aspirin-induced antiplatelet activity or on lowering of the sCD40L concentration.
Cilostazol is a 2-oxo-guinoline derivative with antithrombotic, vasodilator, and antimigrogenic properties. This compound is a potent inhibitor of phosphodiesterase (PDE) 3A, leading to an increase of the intracellular cAMP of platelets because of inhibition of the degradation to AMP (Fig 4). Although the detailed signal transduction pathways remain unclear, increased cAMP can activate cAMP-dependent protein kinase (PKA) in human platelets.\(^{15,19}\) PKA activation induces the phosphorylation of vasodilator-stimulated phosphoprotein, which is closely correlated with inhibition of fibrinogen binding to integrin \(\alpha IIb\beta 3\) and inhibition of platelet aggregation and adhesion.\(^{20}\)

In randomized clinical trials, cilostazol was as effective as ticlopidine\(^{21,22}\) or clopidogrel\(^{23}\) in preventing stent thrombosis after coronary stenting, and triple antiplatelet therapy with aspirin, cilostazol plus either clopidogrel or ticlopidine was more effective in preventing stent thrombosis than aspirin plus clopidogrel or ticlopidine.\(^{16}\) Therefore, ticlopidine was more effective in preventing stent thrombosis after percutaneous coronary intervention: A randomized controlled trial.\(^{160}\) We hypothesized that a triple regimen might result in additional suppression of platelet aggregation and sCD40L, and improve the aspirin and clopidogrel responsiveness in high-risk atherothrombotic patients. Our data from the present study clearly show that adding cilostazol to the standard regimen additionally suppressed ADP-induced platelet aggregation and significantly decreased the rate of low responders to clopidogrel.

Although the exact mechanism of the beneficial effect of adding cilostazol is unclear, our results suggest that cilostazol has a different mode of action (increased cAMP mediated by PDE 3) from that of the P2Y\(_{12}\) receptor inhibition of clopidogrel, and the additional inhibition of ADP-induced platelet aggregation (Fig 4). Supporting results from previous studies show that the addition of cilostazol to an aspirin and clopidogrel regimen results in additional suppression of platelet P-selectin expression.\(^{15}\) Indeed, a 23–35% increase in the inhibition of ADP-induced ex vivo platelet aggregation, with no additive or synergistic effect on AA-induced platelet aggregation, by cilostazol plus aspirin when compared with aspirin alone, has been reported.\(^{24}\) Inhibitory effects on PDE 3A activity correlate with inhibition of platelet aggregation induced by thrombin, collagen, or ADP.\(^{25}\) Taken together, the results of previous studies and the present study suggest that another logical approach to overcoming low responsiveness to clopidogrel might be the addition of cilostazol to the standard dual antiplatelet regimen. Despite the additional antiplatelet effects, cilostazol did not additionally inhibit the release of sCD40L, which is a prothrombotic and proinflammatory biomarker mainly derived from activated platelets in acute coronary syndrome.\(^{26–28}\) Thus further studies are needed to clarify the role of cilostazol in patients with clopidogrel resistance and a high risk for stent thrombosis in the primary PCI setting.

**Study Limitations**

First, the sample size was relatively small, but we found a significant difference in both the PRU value and % inhibition of ADP-induced platelet aggregation between the dual and triple regimens. We also verified the additional benefit of cilostazol for clopidogrel resistance by multivariate regression models. Second, we evaluated ex vivo platelet responsiveness to P2Y\(_{12}\) receptor inhibition based on the VerifyNow P2Y\(_{12}\) test. Ideally, responses would be monitored by light transmission aggregometry using 5 or 20 \(\mu\)mol/L ADP, based on measuring the change in aggregation at baseline and post-drug administration.\(^{29}\) However, ADP-induced light transmission aggregometry is impractical in the primary PCI setting and in a recent validation study the VerifyNow P2Y\(_{12}\) test was shown to be a reliable and sensitive method of monitoring clopidogrel therapy.\(^{30}\)

In conclusion, cilostazol in addition to an aspirin plus clopidogrel regimen led to additional inhibition of ADP-induced platelet activation in the primary PCI setting. This suggests that cilostazol can improve low responsiveness to clopidogrel in patients with acute MI who are undergoing stenting.

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**References**

17. Chen WH, Lee PY, Ng W, Tse HF, Lau CP. Aspirin resistance is associated with a high incidence of myonecrosis after non-urgent


