Original Article

Relationship Between Statin Type and Responsiveness to Clopidogrel in Patients Treated with Percutaneous Coronary Intervention: A Subgroup Analysis of the CILON-T Trial

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Aim: To compare the responsiveness to clopidogrel in patients who were prescribed two different types of statins, atorvastatin vs. rosuvastatin, following percutaneous coronary intervention.

Methods: A total of 915 patients were randomized according to the antiplatelet therapy strategy in the CILON-T trial. In this subgroup analysis, we included patients who took atorvastatin (20 mg/day, $n=295$) or rosuvastatin (10 mg/day, $n=261$) during the entire study period and underwent measurement of the P2Y₁₂ reaction unit (PRU) values at both discharge and six months. We compared the P2Y₁₂ reaction unit (PRU) values at six months and investigated the relationship between the genotypes of cytochrome P450 (CYP) 3A4, 3A5 and 2C19 with the PRU values at six months in both groups.

Results: The baseline characteristics did not differ between the groups. There were no significant differences in the PRU values at discharge (atorvastatin 221.0 ± 87.3 vs. rosuvastatin 217.1 ± 84.7 , $p=0.59$). However, the rosuvastatin group had higher PRU values than the atorvastatin group at six months (atorvastatin 226.4 ± 79.3 vs. rosuvastatin 241.5 ± 88.2 , $p=0.033$). In the genotype analysis, the number of CYP2C19 loss-of-function (LOF) alleles (*2 or *3) was positively associated with a higher PRU value in both statin groups, and there were no significant interactions regarding the PRU values between the number of CYP 2C19 LOF alleles and the type of statin (p for interaction = 0.56). In the multivariate analysis, the use of rosuvastatin was a significant predictor of a PRU value of > 273 (the highest tertile) (OR 1.67, 95% confidence interval 1.05-2.65, $p=0.031$).

Conclusions: Rosuvastatin is associated with high on-treatment platelet reactivity compared with atorvastatin following the coadministration of clopidogrel for six months. Further studies are therefore warranted to clarify the mechanism underlying this relationship.

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Key words: Atorvastatin, Rosuvastatin, Clopidogrel, Platelet function

Introduction

For clopidogrel to exhibit its antiplatelet activity,

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it must be converted to an active metabolite by the liver cytochrome P450 (CYP) system¹. The primary metabolic mediators are CYP2C19, CYP3A and CYP1A2²). A decreased function of CYPs as a result of drug interactions or genetic polymorphisms reduces the antiplatelet effects of clopidogrel, which is associated with poor outcomes after acute myocardial infarction and percutaneous coronary intervention (PCI)²⁻⁵. Previous studies have suggested that the CYP2C19 genotype, a female gender, an old age, dia-

betes mellitus and a decreased renal function are associated with poor responsiveness to clopidogrel⁴⁻⁸.

Statins are frequently administered with clopidogrel in patients with coronary heart disease. Atorvastatin, which inhibits CYP3A, has been reported to suppress the *ex vivo* antiplatelet effects of clopidogrel⁹. However, the results vary depending on the dose of coadministered atorvastatin; thus, the clinical implications of this finding are debatable. Rosuvastatin is primarily metabolized by CYP2C9 into a water-soluble, excretable form^{10, 11}. Its potential drug interaction with clopidogrel has been postulated to be less than that of atorvastatin because it is metabolized by a different pathway. However, few studies have directly compared rosuvastatin and atorvastatin with respect to the statin-clopidogrel interaction^{12, 13}.

The CILON-T trial was designed to compare the clinical efficacy of dual antiplatelet therapy (DAT) and triple antiplatelet therapy (TAT) in patients undergoing drug-eluting stent (DES) implantation¹⁴. This study employs a unique design of stratified randomization of patients according to treatment with statins with different metabolic pathways (CYP3A-metabolized atorvastatin vs. non-CYP3A-metabolized rosuvastatin) in order to exclude potential drug interactions between the statins and clopidogrel. In this subgroup analysis, we compared the degree of *ex vivo* platelet inhibition and changes in lipid profiles in patients receiving equivalent doses of two statins and evaluated the clinical outcomes of patients who underwent DES implantation. We also analyzed the effects of various polymorphisms of the CYP system on posttreatment platelet reactivity in both statin groups.

Methods

The study design of the CILON-T trial has been published elsewhere, and the full protocol is registered at www.clinicaltrials.gov (NCT00776828)¹⁴. A total of 960 consecutive patients who underwent DES implantation were randomized into two groups to receive DAT or TAT. The patients were stratified according to the coadministered statin, with most patients being treated with atorvastatin or rosuvastatin. In the present analysis, we included only those patients ($n=556$) who maintained compliance with atorvastatin (20 mg/day) or rosuvastatin (10 mg/day) treatment throughout the study period and underwent measurement of the P2Y12 reaction unit (PRU) values at both discharge and six months (**Fig. 1**). Patients whose statin regimen or dose was changed during the six-month follow-up were excluded from the analysis.

Laboratory Measurements

The total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) concentrations were measured enzymatically using a TBA-200FR system (Toshiba, Tokyo, Japan) according to the manufacturer's instructions¹⁵. Blood samples for the analysis of lipid profiles were obtained after a 12-hour fast. The lipid levels were measured before randomization as baseline values and analyzed again after six months of study treatment.

The VerifyNowTM assay, a point-of-care assay that employs an entire blood sample, was used according to the manufacturer's instructions¹⁶. The VerifyNowTM P2Y12 assay reports the results in PRUs. This assay mimics turbidimetric aggregation and utilizes disposable cartridges containing 20 μ M of ADP and 22 nM of PGE1. Aggregation testing using ADP as the sole agonist activates P2Y1 and P2Y12 purinergic signaling. Adding PGE1 increases the specificity of the test for P2Y12 signaling^{16, 17}. In a separate channel of the cartridge, in which iso-TRAP is used as an antagonist, the baseline value of the platelet function is obtained, enabling the assessment of platelet inhibition (% inhibition) without having to wean the patient off antiplatelet treatment. We performed the VerifyNowTM P2Y12 assay at discharge and six months after the index procedure.

Genotyping

Genotyping was performed as previously described^{18, 19}. In brief, the genotyping of CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), CYP3A4 (IVS10+12G>A, rs2242480) and CYP3A5*3 (rs776746) SNPs was performed using the TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA). The final volume of polymerase chain reaction (PCR) was 5 μ L, containing 10 ng of genomic DNA and 2.5 μ L of TaqMan Universal PCR Master Mix with 0.13 μ L of 40 $\times$ Assay Mix (Assay identifier: C_25986767_70 for CYP2C19*2, C_27861809_10 for CYP2C19*3, C_26201900_30 for CYP3A4 IVS10+12G>A and C_26201809_30 for CYP3A5*3). The thermal cycling conditions were as follows: 50 $^{\circ}$ C for 2 minutes to activate uracil N-glycosylase and prevent carry-over contamination, 95 $^{\circ}$ C for 10 minutes to activate DNA polymerase, then 45 cycles of 95 $^{\circ}$ C for 15 seconds and 60 $^{\circ}$ C for 1 minute. All PCR analyses were performed in 384-well plates using a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA), and the end point fluorescent readings were obtained using an ABI PRISM 7900HT Sequence Detection System (ABI, Foster City, CA, USA). Duplicate samples and negative controls were

Fig. 1. Flowchart of the patients throughout the study.

included to ensure the accuracy of the genotyping.

Study End Points and Clinical Follow-Up

The incidence of major adverse cardiovascular events (MACEs), including death, nonfatal myocardial infarction (MI), ischemic stroke and ischemic target lesion revascularization (TLR), within six months was compared between the two groups. The cause of death was regarded as being cardiovascular unless there was documented evidence of a clear noncardiovascular cause. Myocardial infarction was defined as a creatinine kinase myocardial band >3 times the upper normal limit. Ischemic stroke was defined as a new focal neurologic deficit of vascular origin lasting at least 24 hours that was proven to be nonhemorrhagic on either CT or MRI scanning. TLR was considered to be clinically driven when it was associated with typical symptoms on clinical assessments, typical signs on a stress test or >70% diameter stenosis on angiographic fol-

low-up. Clinical follow-up was performed at one, three, six and 12 months, and all patients were recommended to undergo follow-up coronary angiography at six months. The investigators followed the patients with either office visits or telephone contact, as necessary. The compliance of the patient with the drug regimen and the occurrence of adverse events were assessed at each visit during the clinical follow-up.

We compared the PRU values and P2Y12 percent inhibition assessed using the VerifyNow™ P2Y12 assay between the two statin groups at discharge and six months after discharge.

Statistical Analysis

The Kolmogorov-Smirnov test was used to test for normality. Comparisons of continuous variables were made using Student's *t*-test or, in cases of a non-normal distribution, the Mann-Whitney *U* test. Categorical variables are presented as numbers or percent-

Table 1. Baseline clinical characteristics of the two groups treated with different statins

	Atorvastatin (<i>n</i> = 295)	Rosuvastatin (<i>n</i> = 261)	<i>p</i>
Age	62.8 ± 9.6	62.0 ± 9.3	0.37
Female	32.3% (141/437)	28.4% (121/426)	0.24
Current smoker	23.1% (68/295)	26.1% (68/261)	0.37
Body mass index	24.9 ± 2.9	24.9 ± 3.2	0.78
Acute coronary syndrome	60.3% (173/287)	52.5% (128/244)	0.08
Past medical history			
Diabetes mellitus	31.2% (92/295)	35.2% (92/261)	0.50
Hypertension	67.1% (198/295)	66.7% (174/261)	0.62
Dyslipidemia	43.4% (128/295)	41.0% (107/261)	0.61
Prior PCI	8.1% (24/295)	7.7% (20/261)	0.88
Prior CABG	2.7% (8/295)	2.7% (7/261)	1.0
Medication at discharge			
Aspirin	100% (295/295)	100% (261/261)	–
Clopidogrel	100% (295/295)	100% (261/261)	–
Cilostazol	50.8% (150/295)	49.8% (130/261)	0.87
Beta-blocker	51.7% (152/294)	54.1% (140/259)	0.61
ACEI	23.1% (68/294)	23.9% (62/259)	0.84
ARB	18.0% (53/294)	22.4% (58/259)	0.20
Calcium channel blocker	25.5% (75/294)	30.9% (80/259)	0.18
Nitrate	39.5% (116/294)	45.2% (117/259)	0.20
Proton pump inhibitor	1.7% (5/294)	1.9% (5/259)	1.0
Medication at 6 months			
Aspirin	99.7% (294/295)	100% (261/261)	1.0
Clopidogrel	97.3% (287/295)	97.7% (255/261)	0.79
Cilostazol	37.6% (111/295)	38.7% (101/261)	0.86
Beta-blocker	60.0% (177/295)	62.1% (162/261)	0.66
ACEI	20.3% (60/295)	21.8% (57/261)	0.68
ARB	20.3% (60/295)	26.4% (69/261)	0.11
Calcium channel blocker	31.9% (94/295)	34.1% (89/261)	0.59
Nitrate	23.1% (68/295)	21.8% (57/261)	0.76
Proton pump inhibitor	1.7% (5/295)	3.4% (9/261)	0.28

The data are presented as the *n* (%) or mean ± SD. Key: ACEI = angiotensin-converting enzyme inhibitor, ARB = angiotensin II receptor blocker.

ages and were compared using the chi-square or Fisher's exact test. The Kruskal-Wallis test was performed to analyze the associations between the CYP genotypes and the on-treatment platelet reactivity (OPR). A logistic regression analysis was performed to identify independent predictors of the highest quartile PRU values at six months. The multivariate model was built according to stepwise variable selection with entry and exit criteria set at the *p* = 0.1 level, and relevant variables were selected from previous studies as candidate variables for our model. The variables included in the multivariable model were as follows: sex, age, diabetes mellitus, renal dysfunction, number of CYP2C19 loss-of-function (LOF) alleles, smoking, use of cilostazol and statin type.

Results

Patients

The VerifyNow™ P2Y12 assay was performed at two different time points: at discharge (*n* = 652, 352 atorvastatin and 330 rosuvastatin) and six months (*n* = 589, 311 atorvastatin and 278 rosuvastatin) after the index procedure. A total of 556 patients (295 atorvastatin and 261 rosuvastatin) underwent measurement of the PRU values at both discharge and six months and were included in the present analysis.

The baseline clinical characteristics of the patients are shown in **Table 1**. There were no significant differences in demographic or clinical characteristics between the two groups. In addition, there were

Fig. 2. On-treatment platelet reactivity at six months in both groups.

(a) P2Y12 reaction units (PRUs) at discharge.

(b) Interval change in the PRU values ($\Delta\text{PRU} = \text{PRU at six months} - \text{PRU at discharge}$).

(c) PRU values at six months.

The data are presented as a whisker plot. The Mann-Whitney U test was used for the analysis.

no differences in medications between the two groups at discharge or six months.

Comparison of Platelet Reactivity between the Two Statin Groups

There were no significant differences in the PRU values at discharge (atorvastatin 221.0 ± 87.3 vs. rosuvastatin 217.1 ± 84.7 , $p=0.59$). However, the interval changes in the PRU values ($\Delta\text{PRU} = \text{PRU at six months} - \text{PRU at discharge}$) were significantly higher in the rosuvastatin group (atorvastatin 5.3 ± 85.6 vs. rosuvastatin 25.1 ± 82.6 , $p=0.0059$), and the rosuvastatin group had higher PRU values than the atorvastatin group at six months (atorvastatin 226.4 ± 79.3 vs. rosuvastatin 241.5 ± 88.2 , $p=0.033$, **Fig. 2**).

The patients treated with DAT exhibited significantly higher OPR than those treated with TAT in both groups (**Supplementary Table 1**). In the atorvastatin group, the patients who received TAT demonstrated an interval change in the PRU value (ΔPRU), while the patients who received DAT did not. However, the interaction between the antiplatelet regimen and type of statin with respect to the ΔPRU was not statistically significant (p for interaction: 0.277, 95% CI -12.5-43.6).

Effects of the Cytochrome P450 Genotype in Both Statin Groups

Genotyping was performed in 387 patients, including 211 patients in the atorvastatin group and 176 patients in the rosuvastatin group. There were no differences in the frequency of CYP2C19*2/*3, CYP3A4 or CYP3A5 between the groups (data not

shown).

The number of CYP2C19 LOF mutations (*2 or *3) was positively associated with a higher PRU value at six months in both the atorvastatin ($p=0.078$) and rosuvastatin ($p=0.013$) groups (**Fig. 3a**). The CYP3A4 and 3A5 genotypes were not associated with the PRU values in either group (**Fig. 3b** and **3c**). We found no interactions between the number of CYP2C19 LOF alleles and the type of statin with respect to the PRU values at six months (p for interaction = 0.56).

Predictors of High Posttreatment Platelet Reactivity

The multivariate analysis revealed the following to be independent predictors of a highest tertile PRU value (>273 units) at six months after DES implantation: the presence of the CYP2C19 LOF allele, dual antiplatelet therapy (vs. triple antiplatelet therapy), female sex, age and rosuvastatin (vs. atorvastatin). These results are summarized in **Table 2**.

Comparison of Lipid Profiles between the Patients Given Two Different Statins in Addition to Clopidogrel

The levels of total cholesterol, triglycerides, LDL cholesterol and HDL cholesterol were compared between the two groups. The baseline lipid profiles showed no differences between the groups. At six months, the rosuvastatin group had lower LDL levels than the atorvastatin group (atorvastatin 66.8 ± 20.5 mg/dL vs. rosuvastatin 62.0 ± 21.6 mg/dL, $p=0.01$). There were no differences in the interval change in the lipid profile, although the LDL levels at six months were lower in the rosuvastatin group than in the ator-

Fig. 3. On-treatment platelet reactivity in both statin groups at six months according to the CYP450 genotype. Atorvastatin ($n=241$) and rosuvastatin ($n=213$).

(a) The association between the P2Y₁₂ reaction unit (PRU) values and the number of CYP2C19 loss-of-function alleles (*2 (rs4244285) or *3 (rs4986893)).

(b) The association between the PRU values and the CYP3A4 (IVS10 + 12G > A, rs2242480) genotype.

The association between the PRU values and the CYP3A5 (*3, rs776746) genotype.

The data are presented as a whisker plot.

The Kruskal-Wallis test and Mann-Whitney U test were used for the analysis.

vastatin group (Table 3). There were no differences in the percentage of patients who maintained an LDL level below 100 mg/dL over the six-month period (atorvastatin 94.2% vs. rosuvastatin 95.1%, $p=0.70$).

The patients treated with TAT showed more favorable triglyceride and HDL profiles than those treated with DAT in both statin groups (Supplementary Table 2).

Clinical Outcomes at Six Months in the Patients Treated with two Different Statins in Addition to Clopidogrel

Table 4 summarizes the clinical outcomes observed in the two statin groups. There were no significant differences between the two groups in the incidence of MACE (atorvastatin 9.8% vs. rosuvastatin 10.3%, $p=0.89$) or any of the components of MACE (cardiac death, MI, ischemic stroke and TLR) at six months.

Table 2. Predictors of high on-treatment platelet reactivity (>273 units) at six months after DES implantation

	OR	95% CI	<i>p</i>
Female (vs. male)	2.26	1.38-3.73	0.001
DAT (vs. TAT)	2.69	1.66-4.35	<0.0001
Number of CYP2C19 loss-of-function alleles, each increase	1.81	1.29-2.54	0.001
Age, each year increase	1.05	1.02-1.08	0.001
Rosuvastatin (vs. atorvastatin)	1.67	1.05-2.65	0.031

Key: DAT=dual antiplatelet therapy (aspirin + clopidogrel), TAT=triple antiplatelet therapy (aspirin + clopidogrel + cilostazol).

Table 3. Lipid profiles at discharge and six months in the two statin groups

	Atorvastatin (<i>n</i> =295)	Rosuvastatin (<i>n</i> =261)	<i>p</i>
At discharge			
Total cholesterol, mg/dL	175.9 ± 41.0 (295/295)	175.6 ± 42.1 (424/426)	0.36
LDL, mg/dL	105.0 ± 35.9 (285/295)	101.8 ± 34.7 (249/261)	0.30
TG, mg/dL	141.9 ± 80.4 (290/295)	138.9 ± 80.0 (257/261)	0.66
HDL, mg/dL	42.8 ± 11.0 (290/295)	43.7 ± 12.1 (256/261)	0.37
At 6 months			
Total cholesterol, mg/dL	131.2 ± 26.1 (292/295)	129.0 ± 27.9 (256/261)	0.35
LDL, mg/dL	66.8 ± 20.5 (275/295)	62.0 ± 21.6 (247/261)	0.01
TG, mg/dL	111.1 ± 52.9 (276/295)	116.3 ± 60.7 (249/261)	0.29
HDL, mg/dL	44.7 ± 11.1 (276/295)	44.9 ± 11.4 (250/261)	0.86

Key: LDL=low-density lipoprotein, TG=triglyceride, HDL=high-density lipoprotein.

Discussion

In the present analysis of data from the CILON-T trial, atorvastatin (20 mg/day) did not attenuate the *ex vivo* antiplatelet efficacy of clopidogrel in patients treated with DES implantation or change the clinical outcomes after six months compared with rosuvastatin (10 mg/day).

Atorvastatin and clopidogrel have CYP3A in common in their metabolic pathways. There is concern regarding potential drug interactions that could attenuate the efficacy of clopidogrel. A previous study demonstrated that atorvastatin attenuates the antiplatelet activity of clopidogrel in a dose-dependent manner⁹. However, there is debate about the clinical significance of this potential clopidogrel-atorvastatin interaction²⁰⁻²³. Therefore, in this analysis, we compared rosuvastatin with atorvastatin because rosuvastatin exhibits minimal hepatic metabolism, and its clearance is not significantly dependent on CYP3A.

However, intriguingly, the rosuvastatin group had higher levels of OPR at six months than the atorvastatin group, and the prescription of rosuvastatin was found to be a risk factor predicting a high OPR level (>273 PRU), which, in Koreans, has been shown

Table 4. Clinical outcomes during follow-up in the two statin groups

	Atorvastatin (<i>n</i> =295)	Rosuvastatin (<i>n</i> =261)	<i>p</i>
MACE	29 (9.8%)	27 (10.3%)	0.89
Death from any cause	0	0	–
Cardiac death	0	0	–
Nonfatal MI	1 (0.3%)	2 (0.8%)	0.60
Stent thrombosis	0	1 (0.4%)	0.47
Ischemic stroke	3 (1.0%)	3 (1.1%)	1.0
TLR	25 (8.5%)	23 (8.8%)	1.0

Key: MACE=major adverse cardiac event, MI=myocardial infarction, TLR=target lesion revascularization.

to be associated with poor clinical outcomes after PCI^{6, 24-26}. This result is compatible with the findings of a previous study that showed a less attenuated ADP-induced P-selectin expression and degree of platelet aggregation in a rosuvastatin group than in an atorvastatin group¹³.

We do not have a clear explanation for the higher values observed in the rosuvastatin group compared to the atorvastatin group. However, the potential mecha-

nisms are as follows. The long-term use of atorvastatin may induce CYP3A4 or 2C19. A recent study that profiled the induction of the CYP450 activity in human hepatocytes by statins using a new liquid chromatography technique, a tandem mass spectrometry cocktail assay, showed that all statins, except pravastatin, induce the P450 activity to some degree, approximately in the order of atorvastatin > simvastatin > lovastatin > rosuvastatin. The inducibility of P450s occurred in the order of CYP2C8 > CYP3A4 > CYP2C9 > CYP2B6 > CYP2C19 $\approx$ CYP2D6 > CYP1A2²⁷⁾. The higher potential of atorvastatin to induce the CYP450 system may play a role in potentiating the conversion of clopidogrel to its active metabolite and hence its antiplatelet effects. Our results appear to be contradictory to those of a recent report by Park *et al.*²⁸⁾. In that study, PCI-treated patients ($n=50$) with HPR were enrolled during the chronic administration of atorvastatin (10 mg/day) and clopidogrel (75 mg/day) (≥ 6 months). The subjects were randomly assigned to receive 15 days of therapy with a non-CYP3A4-metabolized statin, either rosuvastatin (10 mg/day) or pravastatin (20 mg/day). Park *et al.* showed that the degree of platelet aggregation is reduced and the prevalence of HPR decreases after switching from atorvastatin to a non-CYP3A4-metabolized statin. We believe that the differences between these two studies in the dose and duration of rosuvastatin treatment may explain these discrepant results. Patients who exhibit HPR during the chronic administration of atorvastatin and clopidogrel may have weak CYP3A4 activity, even after the chronic induction of CYP3A4 by atorvastatin. These patients may constitute a highly selected population that is prone to exhibiting a good response to a short-term switch to a non-CYP3A4-statin. In addition, the dose of rosuvastatin (10 mg/day) was more potent than that of atorvastatin (10 mg/day)²⁹⁾. Statins themselves have inhibitory effects on platelet activation, and more potent statin treatment may influence platelet function test results^{30, 31)}.

We found no interactions between the CYP2C19 genotype and type of statin in terms of the PRU values at six months. This implies that the statin type exhibits little association with the effects of the CYP2C19 genotype on clopidogrel metabolism. Unknown factors that directly affect the metabolic activity of the CYP450 system irrespective of the CYP2C19 genotype may play a role in the different levels of platelet reactivity observed after clopidogrel therapy between atorvastatin and rosuvastatin users. Our results suggest a new hypothesis regarding the mechanisms of drug interactions between clopidogrel

and statins, although further studies are needed to confirm our findings.

This study revealed that age, gender, the CYP2C19 genotype and the antiplatelet regimen are predictors of a high level of OPR at six months after PCI. The patients receiving DAT had a greater risk of high OPR than those receiving TAT (odds ratio 2.69, 95% confidence interval 1.66-4.35, $p < 0.0001$), and the antiplatelet regimen did not interact with the statin type in predicting a high OPR level, which corresponds to the primary result of the CILON-T trial¹⁴⁾.

In terms of lipid profiles, the patients in the rosuvastatin (10 mg/day) group exhibited no differences in the interval change in the level of LDL compared with those in the atorvastatin (20 mg/day) group over the six-month treatment period, and the percentage of patients achieving the NCEP ATP III LDL goals did not differ between the two groups. More than 90% of the patients achieved their target LDL goal in both groups, which is higher than the rate reported in a previous report on patients with a different ethnic background³²⁾, perhaps because, in our study, the baseline LDL levels were not high and remained well controlled with conventional doses of both statins. We also confirmed that the administration of cilostazol therapy in addition to a statin had favorable effects on the HDL and triglyceride profiles, as suggested by previous studies^{33, 34)}.

In summary, the use of atorvastatin (20 mg/day) did not attenuate the antiplatelet actions of clopidogrel compared with the use of rosuvastatin (10 mg/day). In contrast, rosuvastatin therapy was more frequently associated with a high OPR level than atorvastatin despite having a similar LDL lowering effect in Koreans. Further studies are therefore warranted to document the effects of high-dose statins in other ethnic groups.

Study Limitations

First, the number of patients was relatively small. Second, the degree of platelet reactivity was measured using a single method, the VerifyNowTM P2Y12 assay. Third, our results cannot be extrapolated to patients treated with different types or doses of statins. Fourth, the findings of this study may be specific to Asian populations in which the distribution of genetic polymorphisms and cutoff levels of the PRU values differ from those observed in other populations. More data in different ethnic cohorts must be accumulated in order to elucidate the true effects of different types of statins.

Conflicts of Interest

None.

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References

- Gurbel PA, Antonino MJ, Tantry US: Recent developments in clopidogrel pharmacology and their relation to clinical outcomes. *Expert Opin Drug Metab Toxicol*, 2009; 5: 989-1004
- Simon T, Verstuyft C, Mary-Krause M, Quteineh L, Drouet E, Meneveau N, Steg PG, Ferrières J, Danchin N, Becquemont L; French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction (FAST-MI) Investigators: Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*, 2009; 360: 363-375
- Suh JW, Koo BK, Zhang SY, Park KW, Cho JY, Jang IJ, Lee DS, Sohn DW, Lee MM, Kim HS: Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel. *CMAJ*, 2006; 174: 1715-1722
- Collet JP, Hulot JS, Pena A, Villard E, Esteve JB, Silvain J, Payot L, Brugier D, Cayla G, Beygui F, Bensimon G, Funck-Brentano C, Montalescot G: Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: a cohort study. *Lancet*, 2009; 373: 309-317
- Bates ER, Lau WC, Angiolillo DJ: Clopidogrel-drug interactions. *J Am Coll Cardiol*, 2011; 57: 1251-1263
- Park KW, Jeon KH, Kang SH, Oh IY, Cho HJ, Lee HY, Kang HJ, Park SK, Koo BK, Oh BH, Park YB, Kim HS: Clinical outcomes of high on-treatment platelet reactivity in Koreans receiving elective percutaneous coronary intervention (from results of the CROSS VERIFY study). *Am J Cardiol*, 2011; 108: 1556-1563
- Cuisset T, Gaborit B, Dubois N, Quilici J, Loosveld M, Beguin S, Loundou AD, Moro PJ, Morange PE, Alessi MC, Dutour A, Bonnet JL: Platelet reactivity in diabetic patients undergoing coronary stenting for acute coronary syndrome treated with clopidogrel loading dose followed by prasugrel maintenance therapy. *Int J Cardiol*, 2012 [Epub ahead of print]
- Alexopoulos D, Xanthopoulou I, Tsigkas G, Damelou A, Theodoropoulos KC, Kassimis G, Chouchoulis K, Davlourous P, Chiladakis J, Hahalis G: Predictors of high on-treatment platelet reactivity early after clopidogrel loading in ST-elevation myocardial infarction. *Cir J*, 2012; 76: 2183-2187
- Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, Tait AR, Carville DG, Guyer KE, Bates ER: Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug-drug interaction. *Circulation*, 2003; 107: 32-37
- White CM: A review of the pharmacologic and pharmacokinetic aspects of rosuvastatin. *J Clin Pharmacol*, 2002; 42: 963-970
- McTaggart F: Comparative pharmacology of rosuvastatin. *Atheroscler Suppl*, 2003; 4: 9-14
- Poyet R, Cuisset T, Frere C, Quilici J, Gaborit B, Moro PJ, Camoin L, Morange PE, Bonnet JL, Alessi MC: Comparison of rosuvastatin and atorvastatin on clopidogrel response and lipidic and inflammatory parameters after coronary stenting for acute coronary syndrome: the prospective, randomized OSCAR study (optimal statin therapy with clopidogrel after coronary revascularisation). *Thromb Res*, 2010; 126: e397-399
- Malmstrom RE, Ostergren J, Jorgensen L, Hjemdahl P: Influence of statin treatment on platelet inhibition by clopidogrel - a randomized comparison of rosuvastatin, atorvastatin and simvastatin co-treatment. *J Intern Med*, 2009; 266: 457-466
- Suh JW, Lee SP, Park KW, Lee HY, Kang HJ, Koo BK, Cho YS, Youn TJ, Chae IH, Choi DJ, Rha SW, Bae JH, Kwon TG, Bae JW, Cho MC, Kim HS: Multicenter Randomized Trial Evaluating the Efficacy of Cilostazol on Ischemic Vascular Complications After Drug-Eluting Stent Implantation for Coronary Heart Disease. *J Am Coll Cardiol*, 2011; 57: 280-289
- Cho YK, Hur SH, Han CD, Park HS, Yoon HJ, Kim H, Nam CW, Kim YN, Kim KB, Park NH, Park HJ: Comparison of Ezetimibe/Simvastatin 10/20 mg Versus Atorvastatin 20 mg in Achieving a Target Low Density Lipoprotein-Cholesterol Goal for Patients With Very High Risk. *Korean Circ J*, 2011; 41: 149-153
- Angiolillo DJ, Capranzano P, Goto S, Aslam M, Desai B, Charlton RK, Suzuki Y, Box LC, Shoemaker SB, Zenni MM, Guzman LA, Bass TA: A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: results of the OPTIMUS-2 study. *Eur Heart J*, 2008; 29: 2202-2211
- Fox SC, Behan MW, Heptinstall S: Inhibition of ADP-induced intracellular Ca²⁺ responses and platelet aggregation by the P2Y₁₂ receptor antagonists AR-C69931MX and clopidogrel is enhanced by prostaglandin E₁. *Cell Calcium*, 2004; 35: 39-46
- Park KW, Park JJ, Lee SP, Oh IY, Suh JW, Yang HM, Lee HY, Kang HJ, Cho YS, Koo BK, Youn TJ, Chae IH, Choi DJ, Oh BH, Park YB, Kim HS: Cilostazol attenuates on-treatment platelet reactivity in patients with CYP2C19 loss of function alleles receiving dual antiplatelet therapy: a genetic substudy of the CILON-T randomised controlled trial. *Heart*, 2011; 97: 641-647
- Oh IY, Park KW, Kang SH, Park JJ, Na SH, Kang HJ, Koo BK, Jeong YH, Hwang JY, Kwak CH, Park Y, Hwang SJ, Ko YG, Shin DJ, Jang Y, Kim HS: Association of cytochrome P450 2C19*2 polymorphism with clopidogrel response variability and cardiovascular events in Koreans treated with drug-eluting stents. *Heart*, 2012; 98: 139-144

- 20) Saw J, Brennan DM, Steinhubl SR, Bhatt DL, Mak KH, Fox K, Topol EJ; CHARISMA Investigators: Lack of evidence of a clopidogrel-statin interaction in the CHARISMA trial. *J Am Coll Cardiol*, 2007; 50: 291-295
- 21) Wenaweser P, Windecker S, Billinger M, Cook S, Togni M, Meier B, Haerberli A, Hess OM: Effect of atorvastatin and pravastatin on platelet inhibition by aspirin and clopidogrel treatment in patients with coronary stent thrombosis. *Am J Cardiol*, 2007; 99: 353-356
- 22) Serebruany VL, Midei MG, Malinin AI, Oshrine BR, Lowry DR, Sane DC, Tanguay JF, Steinhubl SR, Berger PB, O'Connor CM, Hennekens CH: Absence of interaction between atorvastatin or other statins and clopidogrel: results from the interaction study. *Arch Intern Med*, 2004; 164: 2051-2057
- 23) Brophy JM, Babapulle MN, Costa V, Rinfret S: A pharmacoepidemiology study of the interaction between atorvastatin and clopidogrel after percutaneous coronary intervention. *Am Heart J*, 2006; 152: 263-269
- 24) Ko YG, Suh JW, Kim BH, Lee CJ, Kim JS, Choi D, Hong MK, Seo MK, Youn TJ, Chae IH, Choi DJ, Jang Y: Comparison of 2 point-of-care platelet function tests, VerifyNow Assay and Multiple Electrode Platelet Aggregometry, for predicting early clinical outcomes in patients undergoing percutaneous coronary intervention. *Am Heart J*, 2011; 161: 383-390
- 25) Jin HR, Yang DH, Kim DI, Seo JS, Jang JS, Kim DK, Kim KH, Seol SH, Nam CW, Hur SH, Kim W, Park JS, Kim YJ, Kim DS: High post-clopidogrel platelet reactivity assessed by a point-of-care assay predicts long-term clinical outcomes in patients with ST-segment elevation myocardial infarction who underwent primary coronary stenting. *Int J Cardiol*, 2012 [Epub ahead of print]
- 26) Ahn SG, Lee SH, Yoon JH, Kim WT, Lee JW, Youn YJ, Ahn MS, Kim JY, Yoo BS, Yoon J, Choe KH: Different prognostic significance of high on-treatment platelet reactivity as assessed by the VerifyNow P212 assay after coronary stenting in patients with and without acute myocardial infarction. *JACC Cardiovasc Interv*, 2012; 5: 259-267
- 27) Feidt DM, Klein K, Hofmann U, Riedmaier S, Knobloch D, Thasler WE, Weiss TS, Schwab M, Zanger UM: Profiling induction of cytochrome p450 enzyme activity by statins using a new liquid chromatography-tandem mass spectrometry cocktail assay in human hepatocytes. *Drug Metab Dispos*, 2010; 38: 1589-1597
- 28) Park Y, Jeong YH, Tantry US, Ahn JH, Kwon TJ, Park JR, Hwang SJ, Gho EH, Bliden KP, Kwak CH, Hwang JY, Kim S, Gurbel PA: Accelerated platelet inhibition by switching from atorvastatin to a non-CYP3A4-metabolized statin in patients with high platelet reactivity (ACCEL-STATIN) study. *Eur Heart J*, 2012; 33: 2151-2162
- 29) Law MR, Wald NJ, Rudnicka AR: Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *BMJ*, 2003; 326: 1423
- 30) Mobarrez F, He S, Bröijersen A, Wiklund B, Antovic A, Antovic J, Egberg N, Jörneskog G, Wallén H: Atorvastatin reduces thrombin generation and expression of tissue factor, P-selectin and GPIIIa on platelet-derived microparticles in patients with peripheral arterial occlusive disease. *Thromb Haemost*, 2011; 106: 344-352
- 31) Mosheimer BA, Kaneider NC, Feistritz C, Djanani A, Sturn DH, Patsch JR, Wiedermann CJ: CD40-ligand-dependent induction of COX-2 gene expression in endothelial cells by activated platelets: inhibitory effects of atorvastatin. *Blood Coagul Fibrinolysis*, 2005; 16: 105-110
- 32) Clearfield MB, Amerena J, Bassand JP, Hernández García HR, Miller SS, Sosef FF, Palmer MK, Bryzinski BS: Comparison of the efficacy and safety of rosuvastatin 10 mg and atorvastatin 20 mg in high-risk patients with hypercholesterolemia--Prospective study to evaluate the Use of Low doses of the Statins Atorvastatin and Rosuvastatin (PULSAR). *Trials*, 2006; 7: 35
- 33) Lee TM, Su SF, Hwang JJ, Tseng CD, Chen MF, Lee YT, Wang SS: Differential lipogenic effects of cilostazol and pentoxifylline in patients with intermittent claudication: potential role for interleukin-6. *Atherosclerosis*, 2001; 158: 471-476
- 34) Tani T, Uehara K, Sudo T, Marukawa K, Yasuda Y, Kimura Y: Cilostazol, a selective type III phosphodiesterase inhibitor, decreases triglyceride and increases HDL cholesterol levels by increasing lipoprotein lipase activity in rats. *Atherosclerosis*, 2000; 152: 299-305

Supplementary Table 1. PRU values at discharge and six months according to the antiplatelet regimen in the two statin groups

	Atorvastatin			Rosuvastatin		
	TAT	DAT	<i>p</i>	TAT	DAT	<i>p</i>
At discharge	209.4 ± 94.1	235.0 ± 77.3	0.011	194.5 ± 84.3	236.7 ± 80.4	<0.0001
Interval change	-4.6 ± 92.6	14.9 ± 76.6	0.049	23.9 ± 94.2	27.3 ± 69.9	0.74
At 6 months	204.7 ± 79.5	249.9 ± 72.6	<0.0001	217.1 ± 93.6	264.0 ± 76.3	<0.0001

Key: TAT = triple antiplatelet therapy, DAT = dual antiplatelet therapy, PRU = P2Y12 reaction unit.
The Mann-Whitney *U* test was used for the analysis.

Supplementary Table 2. Lipid profiles after six months of statin therapy according to the antiplatelet regimen (DAT vs. TAT), demonstrating the effects of cilostazol on the TG and HDL levels

	Atorvastatin			Rosuvastatin		
	TAT	DAT	<i>p</i>	TAT	DAT	<i>p</i>
Total cholesterol, mg/dL	132.3 ± 25.8	130.6 ± 26.3	0.57	129.4 ± 25.0	128.0 ± 30.9	0.71
LDL, mg/dL	66.0 ± 20.1	67.9 ± 21.0	0.45	61.9 ± 22.6	61.7 ± 20.6	0.93
TG, mg/dL	104.0 ± 48.0	118.8 ± 56.5	0.02	108.3 ± 51.9	123.7 ± 67.6	0.04
HDL, mg/dL	46.7 ± 10.8	42.9 ± 11.1	0.004	46.6 ± 11.8	43.2 ± 10.9	0.02

Key: LDL = low-density lipoprotein, TG = triglyceride, HDL = high-density lipoprotein.