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Antiplatelet Effect of Clopidogrel Can Be Reduced by Calcium-Channel Blockers

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Purpose: Clopidogrel is metabolized by the hepatic cytochrome P450 (CYP) system into its active thiol metabolite. CYP3A4 is involved in the metabolism of both clopidogrel and dihydropyridine calcium channel blockers (CCBs). A few reports have suggested an inhibitory interaction between CCBs and clopidogrel. Accordingly, the aim of this study was to determine the effect of CCBs on the antiplatelet activity of clopidogrel by serial P2Y12 reaction unit (PRU) measurements. **Materials and Methods:** We assessed changes in antiplatelet activity in patients receiving both clopidogrel and CCBs for at least 2 months prior to enrollment in the study. The antiplatelet activity of clopidogrel was measured by VerifyNow P2Y12 assay in the same patient while medicated with CCBs and at 8 weeks after discontinuation of CCBs. After discontinuation of the CCBs, angiotensin receptor blockers were newly administered to the patients or dosed up for control of blood pressure. **Results:** Thirty patients finished this study. PRU significantly decreased after discontinuation of CCBs (238.1±74.1 vs. 215.0±69.3; p=0.001). Of the 11 patients with high post-treatment platelet reactivity to clopidogrel (PRU≥275), PRU decreased in nine patients, decreasing below the cut-off value in seven of these nine patients after 8 weeks. Decrease in PRU was not related to CYP2C19 genotype. **Conclusion:** CCBs inhibit the antiplatelet activity of clopidogrel.

Key Words: Calcium channel blockers, clopidogrel, platelet reactivity unit

INTRODUCTION

Clopidogrel is one of the most widely used antiplatelet drugs in patients with acute coronary artery disease and cerebrovascular disease, especially those receiving coronary or carotid artery stent insertion.[1-4] In these patients, calcium channel blockers (CCBs) are commonly used to manage combined hypertension. Clopidogrel is a prodrug that is metabolized by the hepatic cytochrome P450 (CYP) system into its active thiol metabolite; this active metabolite inhibits platelet activation and recruitment by blocking the adenosine diphosphate (ADP) P2Y12 receptor.[5] Among the various CYP enzymes, CYP1A2, CYP2B6, CYP2C19, and CYP3A4/5 are in-

volved in clopidogrel metabolism.[6] CYP3A4 is also involved in the metabolism of both non-dihydropyridine and dihydropyridine CCBs.[7] A few *ex vivo* studies have suggested an inhibitory interaction between CCBs and clopidogrel.[8-10] In contrast, one study showed that concomitant amlodipine did not impact clopidogrel-mediated platelet inhibition.[11] However, these previous studies were retrospective studies and simply compared patients under CCB medication or not. Other confounding factors that may influence clopidogrel resistance, such as genetic polymorphisms and co-medications, were therefore not controlled. The antiplatelet activity of clopidogrel can be inhibited by commonly used drugs. Atorvastatin and omeprazole have been reported to have a negative impact on the antiplatelet effect of clopidogrel.[12] To clarify the effect of CCBs on the antiplatelet activity of clopidogrel, while controlling for other confounding factors, we designed a prospective study to measure antiplatelet activity twice in the same patients: during CCB medication and after discontinuation of CCBs.

MATERIALS AND METHODS

Patients

We enrolled patients who took clopidogrel (75 mg/day) and CCBs for the management of cerebrovascular disease and hypertension; both drugs were maintained for at least 2 months before study enrollment. Patients who had a past history of adverse responses to angiotensin receptor blockers (ARBs) and failure to control blood pressure after discontinuation of CCBs were excluded. All study participants provided written informed consent before enrollment.

The antiplatelet activity of clopidogrel was measured two times by the VerifyNow P2Y12 assay (Accumetrics, San Diego, CA, USA): at baseline and at 8 weeks. Blood pressure was measured by an oscillometric device at baseline and 8 weeks. After the first antiplatelet activity assay, we adjusted anti-hypertensive medication. We stopped CCBs or switched from CCBs to ARBs in patients who were taking CCBs only. Co-medication with CCBs and ARBs was permitted for up to 2 weeks to control blood pressure. In patients who had been taking both CCBs and ARBs previously, the dose of ARBs was increased and the dose of CCBs was decreased or stopped. After 2 weeks, CCBs were discontinued completely. Drug compliance during the study period was investigated at 8 weeks. The study protocol was approved by the Institutional Review Boards of the participating hospitals.

Platelet reactivity measurement

Venous blood for platelet function testing was collected into 3.2% sodium citrate tubes (Vacutainer, Becton Dickinson Company, Franklin Lakes, NJ, USA) using 21-G needles in a one-off successful attempt from a vein that had not been previously punctured. The initial 3 mL of blood was discarded to reduce procedure-related platelet activation.

The antiplatelet activity of clopidogrel, expressed as P2Y12 reaction units (PRU), was measured with the VerifyNow P2Y12 assay.[13] The VerifyNow P2Y12 turbidimetric optical detection system measures platelet-induced aggregation as an increase in light transmittance. In this assay, prostaglandin E1 is used in addition to ADP to increase intraplatelet cAMP, making the assay sensitive and specific for the ADP-mediated effects of the P2Y12 receptor. When citrate-anticoagulated whole blood is added into the assay device, activated platelets are exposed to fibrinogen-coated microparticles, and agglutination occurs in proportion to the number of available platelet receptors.[14] Agglutination is recorded and reported as PRU. A higher PRU reflects greater ADP-mediated platelet reactivity. All measurements were completed within 2 hours of blood sampling. The cut-off value of high post-treatment platelet reactivity to clopidogrel (HPPR) was defined as PRU≥275.[15]

Genetic analysis

The CYP2C19 status of patients was evaluated using the Seeplex CYP2C19 ACE Genotyping system (Seegene, Seoul, Korea). The Seeplex CYP2C19 ACE Genotyping system is a simple, innovative dual priming oligonucleotide primer-based multiplex polymerase chain reaction system with maximal specificity and sensitivity for detecting two single nucleotide polymorphisms (CYP2C19*2, CYP2C19*3 alleles).[16] Patients were classified as wild-type homozygote (*1/*1 allele), heterozygote (*1/*2, *1/*3), or variant homozygote (*2/*2, *2/*3, *3/*3), based on the CYP2C19 genotype results, corresponding to extensive, intermediate, and poor metabolizers, respectively.[17] We assigned extensive metabolizer genotypes to the good genotype group. Patients who were intermediate or poor metabolizers were assigned to the poor genotype group.

Statistical analysis

Statistical analyses were performed using SPSS 19.0 software for Windows (SPSS Inc., an IBM Company, Chicago,

IL, USA). Normal distribution of interval variables was verified by the Kolmogorov-Smirnov test. Continuous variables were expressed as means±SD and categorical variables were expressed as counts (percentages). We used the paired t-test to determine the significance of differences in platelet reactivity at baseline and after discontinuation of CCBs. Kruskal-Wallis test was performed to determine the significance of differences in platelet reactivity at baseline and after discontinuation of CCBs according to genotype, and Wilcoxon signed rank test was performed to determine the significance of differences between the good and poor genotype groups. One way repeated measure analysis of variance (ANOVA) was performed to determine if there were differences in effect between the good and poor genotype groups and to determine if there were differences in effect according to the type of CCB. The χ^2 test was used to assess the significance of differences in demographic data according to genotype. Kruskal-Wallis test was used to compare numerical data. The level of statistical significance was set to $p<0.05$.

RESULTS

Thirty-four patients were enrolled and 30 patients finished this study. Four patients dropped out because of drug noncompliance and study withdrawal. Two patients continued to take CCBs, while one patient did not take clopidogrel continuously. One patient withdrew consent for the study. The numbers of patients with the CYP2C19 *1/*1, *1/*2, *1/*3, *2/*2, *2/*3, and *3/*3 genotypes were 15 (44.1%), 10 (29.4%), 4 (11.8%), 2 (5.9%), 2 (5.9%), and 1 (2.9%), respectively. CYP2C19 genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium ($p=0.852$ for CYP2C19*2, $p=0.335$ for CYP2C19*3). The characteristics of the enrolled patients are shown in Table 1. Details on medication adjustment were as follows: CCBs were stopped in nine patients, switched to ARBs in 12 patients, and stopped after increasing the dose of ARBs in nine patients. PRU measured after discontinuation of CCBs was significantly lower than that measured at baseline (215.0±69.3 vs. 238.1±74.1, $p=0.001$) (Fig. 1). Based on the cut-off value for PRU of 275, 11 patients (36.7%) were categorized as HPPR at baseline. After 8 weeks, PRU was decreased in 9 of the 11 HPPR patients, and in 7 of them, PRU decreased below 275. Therefore, only four patients remained in the HPPR group after discontinuation of CCBs. PRU was increased in the remaining two patients. PRU was significantly lower after discontinuation of CCBs in the extensive and intermediate genotype groups ($p=0.035$, $p=0.043$), but not in the poor genotype group ($p=0.250$). In subgroup analysis, PRU was significantly lower after discontinuation of CCBs in both the good and poor genotype groups ($p=0.035$, $p=0.021$). However, there was no difference in the effect caused by discontinuation of CCBs according to genotype ($p=0.669$). Also, there was no difference between good and poor genotype when the patients were divided into two

Table 1. Clinical Characteristics of the Patients

Characteristic	n=30
Age, yrs	67.5±8.3
Male	19 (63.3%)
Co-administered CCB medication	
Amlodipine	19 (63.3%)
Benidipine	8 (26.7%)
Others (felodipine, cilnidipine)	3 (10.0%)
ARB medication after adjustment*	
Valsartan	16 (53.3%)
Losartan	4 (13.3%)
Candesartan	4 (13.3%)
Telmisartan	3 (10.0%)
Irbesartan	2 (6.7%)
Genotype	
Extensive	14 (46.7%)
Intermediate	12 (40.0%)
Poor	4 (13.3%)
Duration of administered medication	
Calcium channel blocker (months)	41.6±67.9
Clopidogrel (months)	29.0±27.4

CCB, calcium channel blockers; ARB, angiotensin receptor blockers.
*One patient did not take ARB medication.

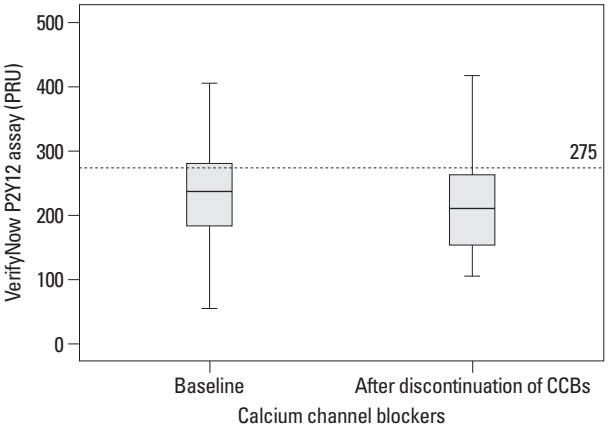

Fig. 1. Box-and-whisker plot showing PRU as determined by the VerifyNow P2Y12 assay in patients at baseline and after discontinuation of CCBs. Cut-off values of high post-treatment platelet reactivity to clopidogrel are indicated by dotted lines. PRU, P2Y12 reaction units; CCBs, calcium channel blockers.

groups (p=0.153). In the extensive genotype group, PRU was decreased in 10 patients and increased in four patients. In the intermediate genotype group, PRU was decreased in 10 patients and increased in two patients. Also, PRU was decreased in three patients and increased one patient in the poor genotype group (Table 2). Both systolic and diastolic pressures were non-significantly increased at 8 weeks compared to those at baseline (systolic blood pressure 124.4±14.6 vs. 131.1±17.3, p=0.067, diastolic blood pressure 76.9±7.1 vs. 78.5±9.9, p=0.412).

DISCUSSION

We found that concomitant use of CCBs and clopidogrel inhibited clopidogrel-related platelet reactivity. This is the first study to investigate changes in clopidogrel-related platelet reactivity in the same patients before and after discontinuation of CCBs. This study design was advantageous in that it allowed for the evaluation of the influences of CCBs while controlling for confounding factors. The conditions of the patients remained the same for the duration of the study period except for adjustment of antihypertensive medication. Therefore, no other factors are likely to have affected the changes in clopidogrel metabolism that we observed.

The interaction between clopidogrel and CCBs has been inconsistently reported. The result of this study is consistent with previous studies.[8,9] Nevertheless, one previous study has suggested that concomitant amlodipine use has no negative impact on clopidogrel mediated platelet inhibition.[11] The previous study was designed to compare PRU between amlodipine and placebo treated groups, and PRU was measured at baseline and 4 weeks. Therein, PRU was not significantly greater in the amlodipine group compared to placebo at 4 weeks. However, PRU increased by 21 units after 4 weeks in the amlodipine treatment group. Although arriving at different conclusions, the present study demonstrated a similar trend to that in this previous study.

Additionally, we also assessed the influence of CCBs on the antiplatelet activity of clopidogrel according to CYP2C19 genotype. A previous study reported that the antiplatelet effect of clopidogrel in patients treated with amlodipine was influenced by CYP3A5 genetic variability.[18] CYP3A5 act as a back-up enzyme for CYP3A4 when CYP3A4 is inhibited. One previous study reported that CYP2C19 polymorphism is associated with a reduced antiplatelet effect of clopidogrel.[19] However, the CYP2C19 genotype did not influence the inhibitory effect of CCBs on the antiplatelet activity of clopidogrel. Discontinuation of CCBs decreased PRU, regardless of CYP2C19 genotype.

In the present study, we defined the cut-off PRU value of HPPR as 275. In a meta-analysis, PRU≥230 was associated with a higher rate of coronary artery disease in patients receiving percutaneous coronary intervention.[20] However, the prevalence of reduced function variants of CYP2C19 is higher in Asians than Caucasians; in Koreans, it has been reported to be as high as 58%.[21] In a recent study, a cut-off PRU value of 275 best predicted thromboembolic events after percutaneous coronary intervention in Koreans. When we used this cut-off value, the proportion of HPPR patients in this study (36.7%) was similar to that reported in a previous study (36.3%).[15] After discontinuation of CCBs, the proportion of HPPR patients decreased from 36.7% to 13.3%.

In seven patients (23.3%), PRU was increased after discontinuation of CCBs. The PRU values in these patients increased by 1 to 63 and from below the cut-off level to over the cut-off level in one patient. We do not have an explanation for the mechanism of PRU increase in these patients. These results might be due to allowable limits of error when

Table 2. Comparison of P2Y12 Reaction Units According to CYP2C19 Genotype

Characteristic	Extensive	Intermediate	Poor	p value
Number of patients	14	12	4	
Age (yrs)	66.9±8.9	69.3±7.9	64.0±7.7	0.574
Male (%)	8 (57.1)	8 (66.7)	3 (75.0)	0.775
Baseline PRU	217.3±57.0	252.8±96.6	266.8±28.5	0.217
Eight wks PRU	196.8±59.6	224.0±85.0	252.0±27.7	0.215
Patients with a decreased PRU (%)	10 (71.4)	10 (83.3)	3 (75.0)	0.843
Patients with an increased PRU (%)	4 (28.6)	2 (16.7)	1 (25.0)	
Duration of CCB medication	47.6±58.6	54.5±98.1	20.8±11.6	0.943
Duration of clopidogrel medication	29.0±30.7	26.1±19.7	37.5±40.1	0.900

PRU, P2Y12 reaction unit; CCB, calcium channel blocker.

repeating the measurement, considering the small increment. As well, the CYP3A5 genotype of the patients could be related to these effects.

Although mean systolic blood pressure was within the normal range before and after medication adjustment with no significant differences between baseline and 8 weeks, there was an increase in systolic blood pressure after changing antihypertensive drugs. It is possible that an increase in systolic blood pressure could increase the risk of cardiovascular disease.[22] A previous study showed that the extent to which blood pressure was lowered was associated with secondary prevention of cardiovascular disease.[23] Therefore, we recommend that blood pressure should be cautiously controlled when considering whether to stop or switch from CCBs to other anti-hypertensive agents.

The limitations of our study include the small numbers of patients examined and the lack of clinical outcome data. Because of our small sample size, we could not clearly define the effect of CCBs on clopidogrel resistance based on CYP2C19 genotype. In addition, we used several different ARBs to control blood pressure after discontinuing CCBs. Although ARBs have no known effects on clopidogrel metabolism, the same ARB should be used in future studies to control for its possible effects. Further large prospective clinical studies are needed to confirm our findings and to determine the effect of CCBs on clinical outcomes.

In conclusion, concomitant use of CCBs can inhibit the antiplatelet activity of clopidogrel. It may be better to avoid the administration of CCBs to patients on clopidogrel medication.

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