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Calcium-Channel Blockers Reduce the Antiplatelet Effect of Clopidogrel

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Objectives Because of the known CYP3A4 inhibition by calcium-channel blockers (CCBs), we hypothesized that there might be a drug-drug interaction between clopidogrel and dihydropyridines in patients with coronary artery disease.

Background Clopidogrel is activated by CYP3A4, which also metabolizes CCBs of the dihydropyridine class.

Methods Responsiveness to clopidogrel was assessed by the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay and aggregometry in 200 patients with coronary artery disease undergoing percutaneous coronary intervention.

Results The platelet reactivity index (PRI) (in the VASP assay, normal range 69% to 100%) was higher in patients receiving both clopidogrel and CCBs (61%) as compared with patients receiving clopidogrel without CCBs (48%). The absolute difference was 13% (95% confidence interval: 6% to 20%; $p = 0.001$), and the relative difference approached 21%. A decreased platelet inhibition by clopidogrel (PRI >69%) was seen in 40% of patients with concomitant CCB treatment and in 20% of patients without concomitant treatment (chi-square test, $p = 0.008$). Intake of CCB remained an independent predictor of reduced platelet inhibition by clopidogrel after adjustment for cardiovascular risk factors. Adenosine diphosphate-induced platelet aggregation was 30% higher in patients on concomitant CCB treatment compared with patients without CCBs ($p = 0.046$). Moreover, intake of CCBs was associated with adverse clinical outcome. In vitro incubation with CCBs (nimodipine, verapamil, amlodipine, and diltiazem) did not alter the PRI or the adenosine diphosphate–induced platelet aggregation of patients taking clopidogrel. This finding indicates that the negative effect occurs in vivo, conceivably at the level of the CYP3A4 cytochrome.

Conclusions Coadministration of CCBs is associated with decreased platelet inhibition by clopidogrel. (J Am Coll Cardiol 2008;52:1557–63) © 2008 by the American College of Cardiology Foundation

The effect of clopidogrel is not uniform in all patients, and decreased platelet inhibition by clopidogrel is seen in about 20% of patients taking clopidogrel and is associated with an 8-fold increased risk of major adverse cardiac events (1–4). Clopidogrel is metabolized to an active thiol metabolite by the CYP3A4 enzyme (5,6). The variability in the response to clopidogrel has been, at least in part, linked to its metabolism by cytochrome P450 enzymes (2). For example, some lipophilic statins may inhibit the CYP3A4 enzyme, and therefore decrease the formation of the active metabolite of clopidogrel (7). Calcium-channel blockers (CCBs), another frequently used class of cardiovascular drugs, also inhibit CYP3A4 (8–10).

Therefore, we hypothesized that intake of CCBs may be associated with decreased responsiveness to clopidogrel. We performed the vasodilator-stimulated phosphoprotein (VASP) phosphorylation assay and platelet aggregometry. The VASP assay is a fairly new platelet function assay (11–13). It is specific for clopidogrel and other P2Y12 antagonists in the absence of cilostazol (14,15), and it can be used to quantify platelet inhibition by clopidogrel (16). It has been shown that adjusting the clopidogrel loading dose according to the platelet reactivity index (PRI) in the VASP assay improves the clinical outcome in patients with decreased platelet inhibition by clopidogrel (17,18).

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This prospective study aimed to compare the responsiveness to clopidogrel in 200 coronary artery disease (CAD) patients with and without concomitant treatment with CCBs.

Methods

Study design. This was a prospective observational study. Patients were followed up for 6 months. The study protocol was approved by the ethics committee of the Medical University of Vienna in accordance with the Declaration of

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Abbreviations and Acronyms

ADP = adenosine diphosphate
CABG = coronary artery bypass graft
CAD = coronary artery disease
CCB = calcium-channel blocker
HR = hazard ratio
MFI = mean fluorescence intensity
PCI = percutaneous coronary intervention
PG = prostaglandin
PRI = platelet reactivity index
VASP = vasodilator-stimulated phosphoprotein

Helsinki. Written informed consent was obtained from all study participants before study entry. Two hundred patients with CAD undergoing percutaneous coronary intervention (PCI) were enrolled. Patients had been on a regimen of clopidogrel (75 mg/day) therapy for 3 months on average (since at least 1 week before study entry) or had received a 600 mg loading dose of clopidogrel (at least 2 h before study entry). The primary end point was a composite of death from cardiovascular causes, nonfatal myocardial infarction, stent thrombosis, and revascularization (PCI or coronary artery bypass graft [CABG] surgery). During the study, samples of 20 healthy volunteers with a normal platelet function were run in parallel to exclude any potential shifts in the readouts of the platelet function tests.

Blood sampling. Blood samples from patients were obtained from the arterial sheath (6-F) in the catheterization laboratory. Blood samples from volunteers were obtained from an antecubital vein, using a 21-gauge needle.

Analysis of VASP phosphorylation by flow cytometry. To determine the VASP phosphorylation state of whole blood, we used a standardized flow cytometric assay (Platelet VASP, BioCytex, Marseille, France), which is an adaptation of the method of Schwarz et al. (19). Blood samples collected in 3.8% sodium citrate (Vacutainer, Becton Dickinson Biosciences, Vienna, Austria) were incubated in vitro with ADP and/or prostaglandin E1 (PGE1) before fixation. After 10 min, platelets were permeabilized, labeled with a primary monoclonal antibody against serine 239-phosphorylated VASP (clone 16C2) or its isotype, followed by a secondary fluorescein isothiocyanate-conjugated polyclonal goat-antimouse antibody. All procedures were performed at room temperature. Platelet geometric mean fluorescence intensity (MFI) was determined using a flow cytometer (FACSCalibur System, Becton Dickinson Biosciences). The platelet population was identified by its forward and side scatter distribution, and 10,000 platelet events were gated and analyzed for MFI. Platelet reactivity was expressed as the PRI, calculated as: PRI% = [(MFI (PGE1) − MFI (PGE1 + ADP)/MFI (PGE1)] × 100. The ratio is expressed as mean percentage platelet reactivity, which inversely correlates with the clopidogrel effect. The VASP assay was performed within 24 h after blood sampling. Using the test results from 20 healthy volunteers without clopidogrel therapy, the reference value for the assay 69% to 100% was calculated by using a nonparametric percentile method (95%). The coefficient of variation of the assay was <5% for duplicates and for testing the same samples on 2 different days.

In vitro experiment. In 10 additional patients, who were receiving clopidogrel but not CCBs, PRI and aggregometry were measured before and after incubation of blood (10 min, 37°C) with 4 different CCBs: verapamil (Isoptin [90 ng/ml], Abbott, Vienna, Austria), diltiazem (Dilzem [200 ng/ml], Elan Pharma International, Ltd., Athlone, Ireland), amlodipine (40 ng/ml, Sigma Aldrich, Vienna, Austria), and nimodipine (Nimotop [10 ng/ml], Bayer, Vienna, Austria). The concentrations of CCBs are the maximum concentrations in plasma (Cmax) described (20–23).

Aggregometry. Whole blood aggregation was determined using an impedance aggregometer (Multiple Platelet Function Analyzer/Multiplate Analyzer, Dynabyte Medical, Munich, Germany). The system detects the electrical impedance change due to the adhesion and aggregation of platelets on 2 independent electrode-set surfaces in the test cuvette (24,25). A 1:2 mixture of 0.9% NaCl and whole blood anticoagulated with hirudin (200 U/ml, Dynabyte, Munich, Germany) were stirred at 37°C for 3 min in the test cuvettes; adenosine diphosphate (ADP [6.4 μM, Dynabyte Medical, Munich, Germany]) was added, and the increase in electrical impedance was recorded continuously for 5 min. The mean values of the 2 independent determinations are expressed as the area under the curve of the aggregation tracing (24). The reference values for the test are 29 to 118 U (26). The results measured by the Multiplate Analyzer are reproducible with a <6% variability (24).

Sample size estimation and statistical analysis. A sample size calculation was based on the observed mean ± SD (61 ± 17) of the PRI under clopidogrel treatment (27). We calculated that we needed to include 200 patients to be able to detect a 15% relative difference in PRI with a power of 95% and a 2-sided alpha value of 0.05. Normal distribution was tested with the Kolmogorov-Smirnov test. Data are expressed as mean and SEM, SD, or 95% confidence intervals (CIs). Statistical comparisons were performed with the *t* test, the Mann-Whitney *U* test, and the chi-square test. Stepwise multivariable logistic regression analysis was used to estimate possible associations between PRI, platelet aggregation, and use of CCBs. The logistic model included age, serum creatinine levels, diabetes mellitus, arterial hypertension, hypercholesterolemia, previous myocardial infarction, smoking, and use of beta-blockers, statins (lipophilic vs. hydrophilic), antidiabetic agents, and angiotensin-converting enzyme inhibitors. Two-year cumulative incidence rates of composite clinical outcomes were estimated by the Kaplan-Meier method. Stepwise multivariable Cox proportional hazards regression modeling was used to estimate the independent effect of concomitant CCB treatment on clinical outcome. The Cox regression model included age, serum creatinine levels, diabetes mellitus, arterial hypertension, hypercholesterolemia, previous myocardial infarction, smoking, and use of beta-blockers, statins (lipophilic vs. hydrophilic), antidiabetic agents, and