Modifying Clopidogrel Maintenance Doses According to Vasodilator-Stimulated Phosphoprotein Phosphorylation Index Improves Clinical Outcome in Patients With Clopidogrel Resistance

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Background: Despite dual antiplatelet therapy, the rate of major adverse cardiovascular events (MACE) after percutaneous coronary intervention (PCI) remains high. Ex vivo tests of clopidogrel resistance can predict MACE after PCI. The purpose of this study is to evaluate the clinical impact of adjusting phosphorylation analysis in patients with clopidogrel resistance undergoing PCI.

Hypothesis: We hypothesized that VASP-guided clopidogrel maintenance doses, compared to fixed doses, improved clinical outcome.

Methods: This monocentric, prospective, randomized study was performed on 306 patients undergoing PCI. Patients were randomized to a control group (n = 156) and to a vasodilator-stimulated phosphoprotein (VASP)-guided group (n = 150). In the VASP-guided group, patients received adjusted maintenance doses of clopidogrel to obtain platelet reactivity index (PRI) of <50% during 1 year after PCI. The primary endpoint was the rate of MACE. The secondary endpoints were major and minor bleeding.

Results: All patients completed the PCI procedure and 298 patients completed follow-up. The control and VASP-guided groups had similar demographic, clinical, and angiographic characteristics. In the VASP-guided group, PRI was significantly decreased (from 72.1% ± 11.4% to 27.7% ± 8.4%; P = 0.001) in 128 patients (87.1% of all participants). During the 1-year follow-up, 14 MACEs were recorded in the VASP-guided group and 30 MACEs were recorded in the control group (9.3% vs 20.4%, respectively; P = 0.008). There was no difference in the rate of major and minor bleeding in the VASP-guided group compared with the control group (12.9% vs 16.6%; P = 0.06).

Conclusions: Modifying clopidogrel maintenance doses according to platelet reactivity monitoring decreases the rate of MACE after PCI without increasing bleeding in patients with clopidogrel resistance during 1-year follow-up.

Introduction

During the last decade, angioplasty has become the most popular method of coronary revascularization. Since the mid-1990s, stent implantation has been the dominant procedure to reduce the rate of acute occlusion and in-stent restenosis. In addition, dual antiplatelet therapy with aspirin and clopidogrel has greatly decreased the risk of major adverse cardiovascular events (MACE) after percutaneous coronary intervention (PCI). Although the addition of thienopyridines to aspirin is widely implemented, recurrent thrombotic events and in-stent thrombosis still occur, which are associated with significant mortality and morbidity. These clinical findings have put forward concern about antiplatelet-therapy resistance. Aspirin resistance is recognized, and several strategies are recommended. More recently, the concept of biological resistance to clopidogrel has caused much attention. Interindividual variability in platelet response to clopidogrel is known to be large. Poor responders represent between 10% and 40% of patients receiving therapy, depending on the tests and thresholds used.

Several methods have been developed to deal with clopidogrel resistance, of which the most popular strategy is increasing the loading dose (LD) utilized in patients undergoing PCI to 600 mg and 900 mg. Although clopidogrel response is dose-dependent, there is a threshold
to its platelet-inhibitory effect when certain doses are administered. In order to find a better method to tackle clopidogrel resistance, Bonello et al. adjusted the clopidogrel LD according to platelet monitoring using the vasodilator-stimulated phosphoprotein (VASP) index in a multicenter randomized prospective study, and observed that it was safe and significantly improved the clinical outcomes after PCI in patients with clopidogrel resistance. In another study, Bonello et al. also demonstrated that tailoring the clopidogrel LD according to platelet reactivity monitoring decreased the rate of early stent thrombosis (ST) after PCI without increasing bleeding. These 2 studies prove the significance of VASP-guided antiplatelet therapy in clopidogrel-resistant patients. However, the relationship between a clopidogrel maintenance dose (MD) and the rate of MACE after initiation of dual antiplatelet therapy >1 month is still uncertain. In the present study, we investigate the impact of a tailored clopidogrel MD according to platelet reactivity monitoring on the rate of MACE in patients after primary PCI during a 1-year period.

**Methods**

**Patients**

A monocentric, prospective study was undertaken in the cardiology department of the university hospital. The study protocol was in accordance with the Declaration of Helsinki and approved by the local ethics committee of our institution. All patients gave written informed consent before inclusion. Patients were enrolled in the study 1 month after undergoing PCI for one of 3 indications: refractory angina pectoris in addition to optimal medical therapy, silent ischemia on thallium scintigraphy, or non–ST-elevation acute coronary syndrome. Other inclusion criteria were patient age >18 years and <80 years, and platelet reactivity index (PRI) <50%. The exclusion criteria were a history of bleeding diathesis, persistent ST-segment elevation acute coronary syndrome (ACS), elevated levels of cardiac markers, New York Heart Association functional class III or IV, contraindications to antiplatelet therapy, platelet count <100 \times 10^9/L, creatinine clearance <25 mL/minute, use of a glycoprotein IIb/IIIa inhibitor, sudden death, and concurrent severe illness with expected survival <1 year.

**Angioplasty Procedure**

Percutaneous coronary intervention was carried out according to international guidelines, using a standard technique, through the radial route. A drug-eluting stent could be used according to American College of Cardiology/American Heart Association (ACC/AHA) guidelines. An intravenous bolus of unfractionated heparin (100 IU/kg) was administered at the beginning of the procedure. The sheath was removed immediately at the end of the procedure in all cases. Combination usage of 100 mg aspirin and 300 mg clopidogrel LD was administered before PCI. After PCI, the combination administration of 100 mg aspirin and clopidogrel was continued and the MD of clopidogrel fluctuated between 75 and 375 mg for 1 year. For patients with non–ST-segment elevation ACS, anticoagulation with low-molecular-weight heparin was initiated in the intensive care unit before PCI. Glycoprotein IIb/IIIa inhibitors were not used.

**Clopidogrel Modification**

One month after PCI, clopidogrel administration was adjusted according to VASP index to keep PRI <50%. The timing of VASP monitoring was 3, 6, 9, and 12 months after first PRI analysis. Clopidogrel doses were increased in a stepwise manner: After the first PRI monitoring (1 month after PCI), 150 mg clopidogrel was administered if PRI was >50%. At the second PRI monitoring, 3 months later, a dose of 225 mg clopidogrel was given to patients if PRI remained at >50%. Another 75 mg of clopidogrel would be added every 3 months if PRI >50%. At the end of 1 year, the maximum MD of clopidogrel would be 375 mg. If PRI was <25% at that time point, the clopidogrel dose would be decreased to 75 mg daily. If PRI fluctuated between 25% and 50%, the determined dose would be maintained.

**Blood Samples**

Blood samples for PRI analysis were drawn by venipuncture of the antecubital vein. The initial blood drawn was discarded to avoid measuring platelet activation induced by the needle puncture. Blood was collected into a tube containing 3.8% trisodium citrate. The tube was inverted 3 to 5 times for gentle mixing and sent immediately to the hemostasis laboratory.

**VASP Phosphorylation Analysis**

The VASP phosphorylation analysis was performed within 1 hour of blood collection by an experienced investigator using a platelet VASP kit (Becton Dickinson, Franklin Lakes, NJ) according to the manufacturer’s instructions. Briefly, blood samples were incubated in vitro with adenosine diphosphate (ADP) and/or prostaglandin E1 (PGE1) before fixation. Each sample was indirectly immunolabeled by incubation with 16C2 fluorescein isothiocyanate (FITC) followed by staining with a goat antimouse FITC polyclonal reagent (Becton Dickinson, Franklin Lakes, NJ). Flow cytometric analysis was performed using a Coulter EPICS XL cytometer (FACSCalibur, BD, Franklin Lakes, NJ). Platelet population was identified on its forward and side scatter distributions, and 3000 platelet events were gated and analyzed for mean fluorescence intensity (MFI) using EPICS XL software. The MFI corresponding to each experimental condition (ADP, ADP + PGE1) was determined to establish a ratio directly correlated with the VASP phosphorylation state. The ratio, \((\text{MFI}_{\text{PGE1}} - \text{MFI}_{\text{ADP + PGE1}})/\text{MFI}_{\text{PGE1}} \times 100\), is expressed in this study as a platelet reactivity index (PRI) corresponding to a ratio of the VASP phosphorylation of activated platelets vs resting platelets and is expressed as a percentage of platelet reactivity. The intra-assay coefficient of variation was <5% and the interassay coefficient of variation was <8%.

**The Early PCI Procedural Outcome**

Stent thrombosis was classified as subacute when it occurred from the end of the PCI procedure up to 30 days later and was classified as late when it occurred after...
30 days. Subacute and late ST were defined according to the Academic Research Consortium.25

**Clinical Endpoints**

Clinical follow-up was initiated 1 month after PCI. Endpoints were recorded by an investigator who was not aware of the treatment status and clinical characteristics of patients. The primary endpoint was the rate of MACE, which included cardiovascular death, angiographically confirmed ST, recurrent ACS defined by the ACC/AHA guidelines,23 and recurrent revascularization by either coronary angioplasty or bypass surgery. Secondary endpoints, recorded to assess safety, were major and minor bleeding. Major bleeding was defined as intracranial bleeding or clinically overt bleeding associated with a decrease in hemoglobin of 50 g/L, according to the Thrombolysis in Myocardial Infarction (TIMI) criteria.26 Minor bleeding was also defined according to TIMI criteria.26 Drug-therapy compliance was assessed.

The treating physician and the investigators who evaluated the clinical endpoints were blind to the results of platelet testing and to group assignment. Prespecified clinical and laboratory data during hospitalization periods were obtained from hospital charts reviewed by independent research personnel who were unaware of the objectives of the study. Clinical follow-up was conducted by telephone contact or office visits. All clinical events were adjudicated by independent physicians unaware of treatment status of the patients and not involved in the study.

**Statistical Analysis**

Statistical analysis was performed using SPSS version 16.0 software (SPSS Inc., Chicago, IL). Continuous variables are expressed as mean ± SD. Categorical variables are expressed as frequency and percentage. Comparison between categorical variables was performed using the χ² test or Fisher exact test when frequencies were <5. Analysis of variance was used to compare characteristics of quantitative variables. Kaplan-Meier curves were used to assess MACE-free survival. Differences between the curves were tested with a log-rank statistic.

**Results**

**Patients’ Demographic, Biological, and Angiographic Characteristics**

From August 2008 to October 2009, 538 continuous patients admitted to the university hospital cardiology center were prospectively screened for inclusion (Figure 1). In total, 232 patients were not included: 20 met the exclusion criteria, and 212 had a PRI <50% after 1 month MD of clopidogrel; therefore, they were considered good responders and were not included in the study. A total of 306 patients had a PRI >50% and were included and randomized to the control group (n=156) or the VASP-guided group (n=150). Five patients in the control group and 3 patients in the VASP-guided group were lost to follow-up. Ultimately, 151 patients in the control group and 147 patients in the VASP-guided group finished the whole study.

Baseline characteristics are summarized in Table 1. Demographic data and clinical characteristics were similar in the 2 groups. The prevalence of cardiovascular risk factors was similar, including diabetes and current smoking (P = 0.5 and P = 0.6, respectively). The PCI indications were balanced among the 3 inclusion criteria and were similar between the 2 randomized groups. The rates of patients undergoing PCI for non–ST-elevation ACS were similar (20% vs 20%, P = 0.7). The 2 groups did not differ in left ventricular ejection fraction (P = 0.6). The PCI data was also similar including the number and length of drug-eluting stents per patient (P = 0.4 and P = 0.2, respectively).

**Platelet Reactivity Index**

We analyzed post-treatment platelet function using the flow cytometric assessment of VASP phosphorylation at a mean time of 1, 3, 6, 9, and 12 months after the patients were randomized to the 2 groups (Table 2). The baseline PRI after PCI showed no significant difference between the 2 groups (69.3% ± 18% for the control group vs 72.1% ± 11.4% for the VASP-guided group, P = 0.4). In the follow-up at 12 months, PRI in the VASP-guided group had decreased significantly from the baseline (27.7% ± 8.4% vs 72.1% ± 11.4%, P = 0.001). The 12-month PRI in the control group also declined, but did not change significantly (66.4% ± 18.6% vs 69.3% ± 18%, P > 0.05). The intervening PCIs for the 3-month, 6-month, and 9-month follow-ups are also included in Table 2. Compared with the control group, the PRI in the VASP-guided group decreased significantly at each follow-up period (P = 0.03, P < 0.001, P = 0.04, respectively).

In the VASP-guided group, the numbers of patients receiving clopidogrel dose modification were 101 (66.9%,
The rate of early ST was not significantly different between the control group and the VASP-guided group (4.7% vs 3.9%, P = 0.3; Table 3). All ST were recorded within 30 days after PCI. There were 2 cases of acute ST (0.9%) and 6 of subacute ST (3.7%) in the control group. In the VASP-guided group, the rate of acute ST was 0.8% and the rate of subacute ST was 3.2%. During 1 month after PCI, 7 patients (2.3%) underwent PCI again because of recurrent myocardial infarction, 3 in the control group and 4 in the VASP-guided group.

Primary Endpoints During Follow-Up
In the follow-up at 12 months, 3 patients in the VASP-guided group and 5 patients in the control group were lost to follow-up. The loss rate was 7.5%. During follow-up, 30 (19.2%) MACE in the control group and 14 (9.3%) MACE in the VASP-guided group occurred, resulting in a statistically difference between the 2 groups (P = 0.008). The distribution of cardiovascular events is summarized in Table 4.

Kaplan-Meier Analysis
Cumulative survival in the 2 groups (Figure 2) was distinguished by the Kaplan-Meier curve. In the first 6 months, survival was significantly different. But after that, the difference stabilized and did not increase. Fewer patients died in the VASP-guided group.

Secondary Endpoints and Side Effects of Clopidogrel
There were no major hemorrhagic complications in either group (Table 4). Both the major and minor bleeding events between the 2 groups were not significantly different.

Discussion
The present study suggests that modifying clopidogrel MDs according to VASP index improves the clinical outcome in clopidogrel-resistant patients undergoing PCI. This strategy of clopidogrel MD adjustment is safe and is not associated with increased bleeding.
Table 2. PRI in the 2 Groups During 1-Year Follow-Up

<table>
<thead>
<tr>
<th>Groups</th>
<th>PRI (Mean ± SD, %) After Randomized</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline PRI</td>
<td>3 Months</td>
</tr>
<tr>
<td>Control group</td>
<td>69.3 ± 18</td>
<td>56.4 ± 21.9</td>
</tr>
<tr>
<td>VASP-guided group</td>
<td>72.1 ± 11.4</td>
<td>43 ± 10.3</td>
</tr>
</tbody>
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P value 0.4 0.03 <0.001 0.04 <0.001

Abbreviations: PRI, platelet reactivity index; VASP, vasodilator-stimulated phosphoprotein.

Table 3. Early Definite ST During 1-Month Follow-Up After Primary PCI

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Control Group (n = 156)</th>
<th>VASP-Guided Group (n = 150)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute ST, n (%)</td>
<td>2 (0.9)</td>
<td>1 (0.8)</td>
<td>0.25</td>
</tr>
<tr>
<td>Subacute ST, n (%)</td>
<td>6 (3.7)</td>
<td>5 (3.2)</td>
<td>0.12</td>
</tr>
<tr>
<td>Early DST, n (%)</td>
<td>8 (4.7)</td>
<td>6 (3.9)</td>
<td>0.30</td>
</tr>
<tr>
<td>Re-PCI, n (%)</td>
<td>3 (1.9)</td>
<td>4 (2.7)</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Abbreviations: DST, definite stent thrombosis; PCI, percutaneous coronary intervention; ST, stent thrombosis; VASP, vasodilator-stimulated phosphoprotein.

The rates of diabetes mellitus and current smoking are higher in the control group, but the difference is not significant. These 2 demographic characteristics do not affect the clinical outcome.

In cases of clopidogrel resistance, authors have recommended new antiplatelet agents ticlopidine, prasugrel, ticagrelor, or cangrelor. Increasing the LDs of clopidogrel is also recommended. However, ticlopidine increases risk of bleeding or dyspnea. In addition, increasing LDs of clopidogrel does not confer more clinical benefit. In recent years, several studies have been launched focusing on VASP-guided clopidogrel modification in patients on clopidogrel. Vasodilator-stimulated phosphoprotein is an intracellular actin-regulatory protein that is a substrate of both cAMP-dependent and cGMP-dependent protein kinases. Analysis of the VASP phosphorylation ratio can be used for measuring various signal transduction processes, including dephosphorylation following P2Y12 ADP receptor activation and its reversal by P2Y12 antagonists. Bonello et al demonstrated that PRI using VASP monitoring is safe and significantly improves clinical outcome after a 600-mg LD. Furthermore, a tailored 600-mg clopidogrel LD according to PRI also prevents acute and subacute ST in the same patients. In a recent experimental study, Schumacher et al demonstrated that a 50% PRI corresponded to a nearly 90% P2Y12 receptor blockade. So we used 50% PRI as the cutoff point.

Our study shows that the MACE occurred during the first 6 months in the follow-up period. In the latter 6 months of...
follow-up, the difference in the incidence of MACE between the 2 groups remained stable. The results indicate that the effect of clopidogrel in preventing thrombosis occurs primarily within 6 months, which is in accordance with Airoldi’s findings in a prospective observational cohort study.  

We observed no increase in bleeding in the VASP-guided group, despite the use of the clopidogrel MD. This may be because platelet monitoring stratifies the dose of clopidogrel according to the individual response, preventing high-dose usage in patients with good response.

Conclusion
This monocentric, prospective, randomized study demonstrates that tailoring clopidogrel MD according to platelet reactivity measured by PRI is safe and may significantly improve clinical outcome after PCI in patients with clopidogrel resistance.

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References
29. Storey RF, Blieden KP, Patil SB, et al. Incidence of dyspnea and assessment of cardiac and pulmonary function in patients with stable coronary artery disease receiving ticagrelor, clopidogrel,


