Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation

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Received 3 March 2006; revised 21 August 2006; accepted 11 September 2006; online publish-ahead-of-print 27 September 2006

Aims To assess whether low response to clopidogrel influences cardiovascular outcome after coronary stent implantation in a consecutively measured cohort of patients with coronary stent implantation.

Methods and results A total of 379 consecutive patients with symptomatic coronary artery disease (CAD), (stable angina \( n = 206 \)) and acute coronary syndrome, \( n = 173 \) treated with percutaneous coronary stenting were enrolled in this trial. Responsiveness to clopidogrel was assessed by ADP (20 \( \mu \)mol/L)-induced aggregometry at least 6 h (mean 34.8 ± 25.9 h) after administration of a loading dose of 600 mg clopidogrel. Platelet inhibition < 30% was defined as low response to clopidogrel. At 3-month follow-up, the primary outcome of a combined major cardiovascular event including non-fatal myocardial infarction, non-fatal ischaemic stroke, or cardiovascular death was evaluated. Twenty-two patients (5.8%) were classified as low responders. Compared with patients who adequately responded to clopidogrel, a low responder had a significantly higher risk of major cardiovascular events [22.7 vs. 5.6%; odds ratio, 4.9; 95% confidence interval (CI), 1.66–14.96; \( P = 0.004 \)]. After adjustment for other factors influencing cardiovascular outcome, low response to clopidogrel and severe left ventricular dysfunction were independently associated with a major cardiovascular event within 3 months (hazard ratio for low response to clopidogrel, 3.71; 95% CI, 1.08–12.69; \( P = 0.037 \)).

Conclusion Low response to clopidogrel in patients with symptomatic CAD treated by stenting significantly enhances the occurrence of cardiovascular events and death. The evaluation of low response to clopidogrel may help to identify patients at increased risk who may benefit from intensified antiplatelet strategy.

KEYWORDS
Clopidogrel; Antiplatelet drug resistance; Platelets; Aggregation; Coronary artery disease

Introduction
Platelets play a critical role in thrombo-ischaemic complications after percutaneous coronary intervention (PCI). Following PCI, dual antiplatelet therapy with aspirin and clopidogrel leads to greater protection from thrombotic complications than aspirin alone. Although antiplatelet agents reduce ischaemic events in patients with high risk for stent thrombosis, the individual inhibitory effect of both aspirin and clopidogrel shows a large variability. Different methods have been used to investigate antiplatelet effects of clopidogrel: the light transmittance aggregometry (LTA) and flow-cytometric analysis of vasodilator stimulated phosphoprotein (VASP) phosphorylation, among them. Recently, we and others showed that a subgroup of patients undergoing PCI does not adequately respond to clopidogrel and have an increased risk to develop subacute stent thrombosis. Further, a substantial percentage of patients with acute myocardial infarction shows a low response to clopidogrel and subsequently is at increased risk of recurrent cardiovascular events in a 6-month follow-up period. However, prospective studies that validate the clinical significance of clopidogrel hyporesponsiveness in large patient cohorts are not available yet. The present study prospectively evaluates the hypothesis whether low response to clopidogrel predicts ischaemic events in patients treated with coronary stent implantation.

Methods
Study population
From March to August 2006, patients admitted to our clinic for coronary intervention for symptomatic coronary artery disease (CAD) were consecutively recruited for this trial. The study was approved by the local Ethics Committee and signed informed consent was obtained from all patients. Patients with known platelet function disorders or treatment with GPIIb–IIIa inhibitors in the week prior to enrolment were excluded from the study. A loading dose of...
600 mg clopidogrel was given to all patients prior to PCI followed by a daily dose of 75 mg for at least 3 months. All patients received a standard dose of aspirin 100 mg daily before enrolment in the study, unless there were contraindications against aspirin use, e.g. risk of gastrointestinal bleeding and allergic reactions. Patient compliance was assessed by telephone interview and 95.8% of patients were followed 3 months after enrolment.

Blood sampling and platelet aggregation

Patient blood was collected at least 6 h (mean 34.8 ± 25.9 h) after first administration of 600 mg clopidogrel, when maximum platelet inhibition was achieved. Blood samples were collected in 3.8% citrate plasma. Probes were centrifuged at 1000 r.p.m. for 10 min to obtain platelet-rich plasma (PRP) and additionally 10 min at 3500 r.p.m. to recover platelet-poor plasma (PPP). Platelet concentration of PRP was adjusted to 2 × 10^5/L by adding homologous PPP. Per cent platelet aggregation after stimulation with 20 μmol/L adenosine diphosphate (ADP) was assessed with the turbidimetric method using a Chronolog Lumi aggregometer with Aggro-Link Software.

Definition of low response to clopidogrel

We defined low response to clopidogrel if post-treatment aggregation was >70%. The rationale for this value chosen is based on previous data, in which a platelet inhibition of <30% was defined as low response to clopidogrel. Additionally, in a retrospective analysis of distribution of values in the present study, we found the lower bound of the upper quartile of ADP(20 μmol/L)-induced aggregation ~70%. The definition of low response for patients with a platelet reactivity in the upper quartile has been documented by others. Therefore, we considered this practical cutoff value as reasonable to define low response.

Previous events, follow-up, and causes of death

The classification of previous events and follow-up data was made on the basis of medical records and personal interviews and was available for >90% of patients. Causes of death were determined by examination of hospital records, autopsy reports, and medical files of the patients’ general practitioners. Deaths due to cardiovascular causes included sudden deaths and deaths from acute myocardial infarction, CAD, or congestive heart failure.

Statistical methods

Association of low response to clopidogrel with pre-specified primary endpoints including death from cardiovascular causes, non-fatal myocardial infarction, and non-fatal ischaemic stroke was evaluated within a 3-month follow-up.

With a probability of 80% that the study will detect a minimal hazard ratio (HR) of 2.25% for the primary endpoint at a one-sided 5.0% significance level and a presumed low responder rate of up to 10%, we estimated a sample size of 335 patients.

Mean values between two categories were compared with a two-tailed unpaired t-test. The χ^2 test was used for dichotomous analysis of categorical data. Log-rank test (Mantel–Cox) was applied for the evaluation of associations between survival and variables.

Cox regression analysis was performed to compare the association of continuous values of ADP (20 μmol/L)-induced platelet aggregation with combined cardiovascular endpoints. The association of low response to clopidogrel using the pre-defined cutoff value with combined cardiovascular events was tested by an analysis of multivariable Cox proportional hazards survival regression after adjustment for epidemiological factors influencing cardiovascular outcome.

The time-dependent covariate method was used to check the proportional hazard assumption of the model. The HR represents the predicted change in the hazard for a unit increase in the predictor (e.g. a change from normal to low response to clopidogrel). The Kaplan–Meier survival was used to estimate event-free survival and survival of death from cardiovascular causes.

All probability values reported are two-sided, and a value of P < 0.05 was considered to indicate statistical significance. Statistical analysis was done with SPSS software, version 13 for windows (SPSS, Inc., Chicago, IL, USA). Event classification was performed by an investigator blinded for the state of clopidogrel responding.

Results

Baseline characteristics

During the study period, 379 (95.2%) of 398 eligible patients treated by percutaneous coronary stenting were enrolled in this study. Thirty-one patients were deemed ineligible due to platelet function disorders or treatment with GPIIb–IIa inhibitors within 1 week prior to possible enrolment. Of the patients initially assessed for inclusion, 19 patients refused consent. Of the enrolled patients, 16 patients (4.2%) were lost to follow-up. The mean (±SD) age of the remaining 363 patients was 67.5 ± 10.0 years (range, 33–91 years). Detailed characteristics of the patients are listed in Table 1.

The mean (±SD) of ADP (20 μmol/L)-induced aggregation of the tested 379 patients was 34.5 ± 22.6% and the platelet activity of the study population followed a normal distribution. Twenty-two patients (5.8%) showed a low response to clopidogrel (inhibition of platelet aggregation <30%) and were defined as clopidogrel low responders. In univariate analysis (χ^2), patients with acute coronary syndrome more frequently showed a low response to clopidogrel than those with stable angina pectoris elective for coronary stent implantation (81.8 vs. 43.1; P < 0.001) (Table 1).

In this study, patients with a low response to clopidogrel were less frequently hyperlipidaemic (27.3 vs. 62.8; P = 0.001) (Table 1).

Incidence of death and cardiovascular events

Table 2 shows the incidence of cardiovascular outcomes during the 3-month follow-up. A total of 19 patients (5.2%) died, 14 from cardiovascular causes (3.9%); other causes included acute renal failure (one), pneumonia (two), and multiorgan failure (two). Five patients had non-fatal acute myocardial infarction (1.4%) and five patients suffered from non-fatal ischaemic stroke (1.4%) within 3-month follow-up.

Association of platelet aggregation with cardiovascular events

In the Cox regression analysis, continuous values of ADP (20 μmol/L)-induced platelet aggregation were significantly associated with a major cardiovascular event within 3 months [HR, 1.18; 95% confidence interval (CI), 1.02–1.38; P = 0.046].

Low response to clopidogrel and clinical outcomes

Low response to clopidogrel was associated with an increased risk of a major cardiovascular event and increased risk of death from cardiovascular causes after 3 months [composite cardiovascular events: 22.7 vs. 5.6%; odds ratio (OR), 4.9; 95% CI, 1.66–14.96; P = 0.004 and cardiovascular outcomes: 24.9 vs. 6.8%; HR, 4.1; 95% CI, 1.26–13.14; P = 0.02].
mortality: 18.2 vs. 2.9%; OR, 7.36; 95% CI, 2.10–25.75; \( P = 0.002 \) (Table 2).

In univariate analysis, the occurrence of a major cardiovascular event within 3 months was significantly influenced by a low response to clopidogrel \( (P = 0.01) \), severe left ventricular (LV) dysfunction \( (P < 0.001) \), advanced age \( (P = 0.004) \), and treatment with statins \( (P = 0.012) \).

The incidence of death from cardiovascular causes was influenced by a low response to clopidogrel \( (P = 0.007) \), severe LV dysfunction \( (P < 0.001) \), and concomitant treatment of following medications: angiotensin-converting enzyme (ACE)-inhibitors \( (P = 0.006) \) and beta-blockers \( (P = 0.001) \).

Cumulative event-free survival from cardiovascular death increased, depending on response to clopidogrel (Figure 1). Multivariable Cox proportional survival regression identified severe LV dysfunction and low response to clopidogrel and the non-use of ACE-inhibitors as predictors of death from cardiovascular causes (low response to clopidogrel: HR, 10.34; 95% CI, 2.14–49.85; \( P < 0.01 \); severe LV dysfunction: HR: 10.41, 95% CI, 2.92–37.12; \( P < 0.001 \); and concomitant treatment with ACE-inhibitors: HR, 3.94; 95% CI, 1.00–15.47; \( P = 0.05 \)) independently from other epidemiological factors influencing cardiovascular mortality.

Cumulative event-free survival increased with response to clopidogrel in an analysis of combined cardiovascular events.
In the multivariable Cox regression analysis, the occurrence of a major cardiovascular event after coronary stent implantation was significantly influenced by a low response to clopidogrel and severe LV dysfunction (low response to clopidogrel: HR, 3.71; 95% CI, 1.08–12.69; \( P = 0.04 \) and severe LV dysfunction: HR, 3.81; 95% CI, 1.38–10.53; \( P = 0.01 \)) (Table 3).

**Table 3** Results of multivariable Cox regression analysis for composite cardiovascular endpoints adjusted for factors influencing cardiovascular outcome

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard ratio (95.0% CI)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low response to clopidogrel</td>
<td>3.71 (1.08–12.69)</td>
<td>0.04</td>
</tr>
<tr>
<td>LV dysfunction (EF ≤ 30%)</td>
<td>3.81 (1.38–10.53)</td>
<td>0.01</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.39 (0.54–3.54)</td>
<td>0.50</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.71 (0.21–2.43)</td>
<td>0.59</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>0.62 (0.23–1.68)</td>
<td>0.35</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>1.17 (0.42–3.24)</td>
<td>0.77</td>
</tr>
<tr>
<td>Age (&gt;65 years)</td>
<td>2.07 (0.54–7.87)</td>
<td>0.29</td>
</tr>
<tr>
<td>Prior acute coronary syndrome</td>
<td>1.28 (0.49–3.35)</td>
<td>0.61</td>
</tr>
<tr>
<td>Concomitant treatment with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>1.26 (0.33–4.76)</td>
<td>0.74</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>1.84 (0.67–5.03)</td>
<td>0.24</td>
</tr>
<tr>
<td>Statins</td>
<td>2.40 (0.90–6.44)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Discussion**

The principal finding of the current study is that low response to clopidogrel suggests ischemic events in patients after coronary stent implantation. We found that ex vivo analysis of ADP-induced platelet aggregation after coronary stenting identifies patients at high risk of major cardiovascular events (myocardial infarction, stroke, and death) at a 3-month follow-up. The finding of the present study implies that a subpopulation of patients with CAD treated with PCI (~6%) does not adequately respond to clopidogrel and may require an intensified dual antiplatelet therapy.

Recent clinical studies clearly demonstrate the beneficial effects of dual antiplatelet therapy (aspirin plus clopidogrel) after PCI in patients with CAD and strongly support the clinical significance of platelet inhibition. However, major cardiovascular events occurred in ~9% of patients despite dual antiplatelet therapy. Preliminary results seem to indicate that low antiplatelet effect of clopidogrel may lead to a higher risk of developing cardiovascular events. Thus, we evaluated in a prospective study the clinical significance of responsiveness to clopidogrel in a total of 379 consecutive patients and observed an association between ADP (20 \( \mu \)mol/L)-induced platelet aggregation and cardiovascular events. Furthermore, we found that low response to clopidogrel as determined by conventional light aggregometry occurred in ~6% of patients treated with coronary stenting and was associated with poor clinical outcome at 3-month follow-up. However, owing to the small number of clopidogrel low responders, we are aware of a possible under- or overestimation of this effect.

Platelet function is measured ex vivo, in most instances, by LTA. Although this method carries some disadvantages such as limited reproducibility and complex sample preparation, it is currently the most widely used method to test the effect of antiplatelet drugs. In the past, ADP-induced platelet aggregation had been successfully used to evaluate the pharmacokinetics and individual variability of clopidogrel response. Another method to monitor clopidogrel action is the flow-cytometric analysis of VASP phosphorylation. It investigates the specific inhibition of the P2Y\(_{12}\) ADP receptor. Both methods have

(Figure 2). In the multivariable Cox regression analysis, the occurrence of a major cardiovascular event after coronary stent implantation was significantly influenced by a low response to clopidogrel and severe LV dysfunction (low response to clopidogrel: HR, 3.71; 95% CI, 1.08–12.69; \( P = 0.04 \) and severe LV dysfunction: HR, 3.81; 95% CI, 1.38–10.53; \( P = 0.01 \)) (Table 3).
been shown to correlate well.\textsuperscript{19} In the present study, we used ADP-induced aggregation measured by LTA to define low response to clopidogrel. Presently, there is no clear consensus cutoff value to identify low responders to clopidogrel. The authors have used empirically defined cutoff values varying between 10 and 40\% to segregate low responders from responders.\textsuperscript{4,5} We used an arbitrary cutoff value of clopidogrel-dependent platelet inhibition of <30\% and found that ~6\% of patients were classified as low responders in our patient cohort. Current published data show that ~4–11\% of patients treated with conventional doses of clopidogrel do not display adequate antiplatelet response.\textsuperscript{9} In patients with acute myocardial infarction, the percentage of hyporesponders is even higher (up to 25\%).\textsuperscript{8} Thus, our definition of low response to clopidogrel corresponds well with the published data.

The time chosen to measure platelet aggregation is also of importance to define hyporesponsiveness to clopidogrel. A 300 mg loading dose of clopidogrel can only elicit its full antiplatelet effect at 24 h,\textsuperscript{6} in contrast to a 600 mg loading dose, which can achieve its maximum effect after only 4 h.\textsuperscript{20} Thus, we evaluated the degree of platelet inhibition more than 6 h after administration of 600 mg loading dose to reassure that platelet testing was performed when maximal inhibition had been achieved.

Our results suggest that a single measurement of ADP-induced platelet aggregation in patients undergoing coronary stenting can be used to identify patients at high risk of major adverse cardiac events. This finding supports the notion that in a subgroup of patients, the standard post-interventional antiplatelet regimen with a maintenance standard dose of clopidogrel 75 mg daily might not be the optimal dosage to prevent major cardiovascular events. Additionally, in the univariate analysis, we found an association of acute coronary syndrome with low response to clopidogrel. This fact implies that patients with acute coronary syndrome have a significant higher post-treatment platelet activity and might not receive optimal platelet inhibition by a standard dose of clopidogrel. Recently, it was shown that administration of a 600 mg loading dose of clopidogrel in patients already chronically treated with clopidogrel results in a significant additional inhibition of ADP-induced platelet aggregation.\textsuperscript{21} Thus, the degree of platelet inhibition can be at least transiently augmented when higher dosages of clopidogrel are administered. It is currently unclear whether a higher transient maintenance dose during critical, high-risk period (e.g. after PCI) results in pronounced platelet inhibition and improves clinical outcome. Whether an increase in the maintenance dose of clopidogrel enhances clopidogrel responsiveness or an alternative antiplatelet strategy (e.g. prasugrel) improves clinical outcome in coronary stent patients remains to be explored in upcoming trials.

Acknowledgement

The work was supported in part by the Deutsche Forschungsgemeinschaft, the Wilhelm Sander Stiftung and the Fortune-Program of the University of Tübingen.

Conflict of interest: none declared.

References


