A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days

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Introduction

Dual antiplatelet therapy consisting of aspirin and clopidogrel is currently the therapy of choice to prevent thrombosis after percutaneous coronary intervention (PCI).\(^1\,^2\) Clopidogrel is a prodrug that needs to be metabolized to an active metabolite. The active metabolite covalently binds to and irreversibly blocks the P2Y12 platelet ADP receptor.\(^3\) The effect of clopidogrel on platelet function is most commonly assessed by measuring ADP-induced platelet aggregation with optical aggregometry. This method was also used in initial studies on single- and repeated-dose pharmacodynamics.\(^4\,^6\) In these dose-finding studies, the antiaggregatory effects of daily maintenance doses ranging from 10 to 150 mg were studied.\(^5\,^6\) Although, in one study, a trend towards increased inhibition of ADP-induced aggregation with a 150 mg daily maintenance dose was observed when compared with daily doses ranging from 50 to 100 mg, it was assumed that a plateau response is reached with administration of 75 mg once daily.\(^5\) Moreover, with administration of 75 mg once daily, the same degree of inhibition of platelet aggregation was achieved as with ticlopidine 250 mg twice daily, which was the target level of inhibition. On the basis of these results, the currently recommended maintenance dose of clopidogrel (75 mg/day) was chosen for the phase III Clopidogrel vs. Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) trial.

Despite clopidogrel's proven efficacy in reducing thrombotic events, acute or subacute stent thrombosis is still a significant clinical problem that occurs in 1–2% of patients treated. A considerable interindividual variability in response to clopidogrel has been observed after administration of loading doses of clopidogrel and in patients chronically treated with the recommended maintenance dose.\(^8\,^13\) In a significant proportion of patients (10–30%), no or little inhibition of platelet aggregation is achieved with the currently used dosing regimens.\(^8\,^11\) Some data suggest that these patients are at an increased risk of stent thrombosis.\(^8\,^11\)

Pre-treatment with a clopidogrel-loading dose is recommended in patients undergoing PCI.\(^5\) Recently, we showed that administration of a 600 mg dose of clopidogrel in patients already chronically treated with a maintenance dose of 75 mg/day results in a significant additional inhibition of ADP-induced platelet aggregation and surface expression of glycoprotein (GP) IIb/IIIa and P-selectin after...
stimulation with ADP.17 These results also suggest that the antiplatelet effect achieved with the currently recommended maintenance dose can be augmented. In fact, administration of a 150 mg daily maintenance dose is now broadly discussed and occasionally used in clinical practice, although there is still a lack of functional data proving its efficacy. More intense inhibition of platelet aggregation associated with an increased daily maintenance dose may result in fewer ischaemic events after PCI, particularly in high-risk patients.3,18

Here, we report the first randomized trial in which the antiplatelet effects of two different clopidogrel maintenance doses are compared in patients who were treated with PCI. The main hypothesis was that an increase in the clopidogrel daily maintenance dose from 75 to 150 mg results in a more intense inhibition of ADP-induced platelet aggregation.

Methods

Patients

Patients on chronic aspirin therapy, who had been treated with PCI after administration of a 600 mg loading dose of clopidogrel, were eligible for this trial. Patients who underwent PCI due to unstable angina and acute myocardial infarction or those who were haemodynamically unstable were excluded from the trial. Other exclusion criteria included stroke within 3 months, malignancies, active bleeding and bleeding diatheses, oral anticoagulation therapy with a coumarin derivate, recent treatment (≤30 days) with a GP IIb/IIIa antagonist or other antiplatelet drugs (particularly clopidogrel) except for aspirin, platelet count <100 x 10^9/L, serum creatinine >2 mg/dL, and/or liver disease resulting in bilirubin >2 mg/dL. The study protocol was approved by the institutional Ethics Committee, and the patients gave written informed consent for participation.

Randomization, administration of the randomized clopidogrel maintenance dose, and blood sampling

Eligible patients were enrolled into the trial on behalf of the ISAR-CHOICE 2 (Intracoronary Stenting and Antithrombotic Regimen: Choose a High Oral maintenance dose for Intensified Clopidogrel Effect 2) investigators. Flow of ISAR-CHOICE 2 participants is illustrated in Figure 1. Patients received the first randomized clopidogrel maintenance dose within 12 h of PCI. To enable double-blind randomization, capsules that contained either 75 or 150 mg of clopidogrel were prepared. The clopidogrel-containing capsules and the randomization sequence were provided by the pharmacy of the Deutsches Herzzentrum München, Munich, Germany. The randomization sequence was computer generated with a block size of 6. No stratification was used. The study medication was provided by the pharmacy in sealed opaque envelopes. The capsules with the two doses of the study medication appeared similar and had the same weight. Physicians and operators who performed platelet-function testing were blinded as to the actual clopidogrel dose. After PCI, all patients received 100 mg of aspirin twice daily and the capsules with the clopidogrel study medication every morning. A 30-day follow-up visit was arranged to assess the antiplatelet effect of the two different clopidogrel maintenance doses and clinical status. Before discharge, the patients were handed out a sufficient number of clopidogrel-containing capsules necessary for uninterrupted administration. At the 30-day follow-up visit, the remaining clopidogrel-containing capsules were counted to assess compliance with the study protocol. At the 30-day (outpatient) follow-up visit, blood samples were obtained for platelet-function testing 4–6 h after the last intake of the study medication. Peripheral venous blood samples were drawn in a fasting state with a loose tourniquet through a short venous catheter inserted into a forearm vein. A multiple-syringe sampling technique was used and the first 2 mL of blood was discarded. For optical aggregometry, peripheral venous blood was collected in 3.8% citrate. For platelet-function testing with the VerifyNow™ P2Y12 assay, peripheral venous blood was collected in 3.2% citrate (1.8 mL draw plastic Vacutette tubes; Greiner, Monroe, NC, USA).

Aggregometry

Citrated blood samples for aggregometry were processed within 60 min. Platelet aggregation was evaluated by optical aggregometry in platelet-rich plasma (PRP) using a Chrono-log lumi-aggregometer (Probe & go Labordiagnostica, Endingen, Germany) with a constant stirring rate of 1000 r.p.m. at 37 °C. The final platelet count was adjusted to 300 x 10^9/L with autologous platelet-poor plasma. PRP (0% light transmission) and platelet-poor plasma (100% light transmission) served as references. After baseline adjustment, ADP (final concentrations of 5 or 20 μM) was added and aggregation
recorded for 5 min. The analysed parameter was maximal aggregation (%).

**Platelet-function testing with the VerifyNow™ P2Y12 assay**

VerifyNow™ (formerly called Ultegra rapid platelet-function assay; Accumetrics, Inc., San Diego, CA, USA) is a whole-blood, point-of-care assay, which consists of an instrument and a single-use assay device containing the biochemical reagents required to perform an assay. The VerifyNow™ P2Y12 assay has been developed to assess responsiveness to clopidogrel and other P2Y12 antagonists. In addition to 20 μM ADP, 22 nM prostaglandin E1 is incorporated into the VerifyNow™ P2Y12 assay device to suppress intracellular free calcium levels and thereby to reduce the activation contribution from ADP binding to P2Y1 receptors. In a separate channel in which iso-TRAP is used as an agonist, a baseline value (BASE) for platelet function is obtained. The VerifyNow™ P2Y12 assay reports patient results as P2Y12 reaction units (PRU), ‘%inhibition’, and ‘BASE’ in <5 min. %Inhibition is calculated as (1-PRU/BASE)*100. The assay was performed according to the instructions of the manufacturer.

**Endpoints and sample-size calculation**

The primary endpoint of the study was maximal 5 μM ADP-induced platelet aggregation assessed with conventional aggregometry 30 days after PCI. We hypothesized that an increase in the daily maintenance dose from 75 to 150 mg results in a 30% reduction of maximal 5 μM ADP-induced platelet aggregation (mean ± standard deviation from 50 ± 14 to 35 ± 14%). Choosing a power of 95% and a two-sided α-level of 0.05, at least 24 patients in each group were required. Other endpoints were maximal ADP(20 μM)-induced aggregation and PRU and %inhibition reported by the VerifyNow™ P2Y12 assay.

**Statistical analysis**

Data are presented as mean ± SD, counts, or percentages if not otherwise stated. Mean values of continuous variables were compared with Student’s t-test. P-values <0.05 were considered statistically significant.

**Results**

Baseline characteristics and concomitant therapy of the patients according to clopidogrel maintenance dose are presented in **Table 1**. No significant differences were observed. According to Thrombolysis In Myocardial Infarction (TIMI) criteria, two minor and no major bleeding events occurred in each treatment group. During 30 days after PCI, one myocardial infarction occurred in the group treated with 150 mg/day and one target vessel revascularization was required in the group treated with 75 mg/day.

Platelet function was assessed 30 days after PCI in all patients. All patients brought the remaining capsules to the follow-up visit, and in all patients, the number of remaining capsules demonstrated full compliance with the study protocol. The results of platelet-function testing 30 days after PCI are shown in **Figures 2 and 3**. Maximal 5 μM ADP-induced platelet aggregation 30 days after PCI was significantly lower in the group treated with 150 mg/day (45.1 ± 20.9%) than in the group treated with 75 mg/day (65.3 ± 12.1%; P < 0.001). Wilcoxon rank-sum test yielded a P-value of <0.001 [median of maximal 5 μM ADP-induced aggregation (150 mg/day) 26.3 (IQR 16.5–38.8) vs. 50.0 (IQR 38.0–67.5); P < 0.001].

**Table 1** Baseline characteristics of patients and concomitant medication at discharge after PCI according to clopidogrel maintenance dose

<table>
<thead>
<tr>
<th></th>
<th>150 mg/day</th>
<th>75 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>63.0 ± 7.5</td>
<td>65.4 ± 6.9</td>
</tr>
<tr>
<td>Female</td>
<td>3 (9.7)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>89.2 ± 17.4</td>
<td>82.8 ± 9.8</td>
</tr>
<tr>
<td>Height, cm</td>
<td>176.2 ± 6.8</td>
<td>174.8 ± 6.5</td>
</tr>
<tr>
<td>Platelet count, ×10^9/L</td>
<td>217.2 ± 64.7</td>
<td>222.8 ± 44.4</td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td>14 (45.2)</td>
<td>15 (51.7)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>16 (51.6)</td>
<td>14 (48.3)</td>
</tr>
<tr>
<td>Active smokers</td>
<td>3 (9.7)</td>
<td>2 (6.9)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (22.6)</td>
<td>10 (34.5)</td>
</tr>
<tr>
<td>Concomitant medication</td>
<td>31 (100)</td>
<td>29 (100)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>31 (100)</td>
<td>29 (100)</td>
</tr>
<tr>
<td>β-blockers</td>
<td>28 (90.3)</td>
<td>28 (96.6)</td>
</tr>
<tr>
<td>ACE-inhibitors</td>
<td>27 (87.1)</td>
<td>26 (89.7)</td>
</tr>
<tr>
<td>Statins</td>
<td>31 (100)</td>
<td>28 (96.6)</td>
</tr>
</tbody>
</table>

Data presented are mean ± standard deviation or number of patients (percentages). ACE, angiotensin-converting enzyme.

**Figure 2** Maximal aggregation induced by 5 (left) and 20 (right) μM ADP in patients treated with two different clopidogrel daily maintenance doses (150 and 75 mg). Individual data are shown, along with mean (thick lines) and SD (thin lines).
platelet aggregation 46.0 (25th and 75th percentiles: 26.5 and 57.5) in patients treated with 150 mg/day and 66.0 (58.0 and 70.0) in patients treated with 75 mg/day. The VerifyNow™ P2Y12 assay also indicated a higher degree of platelet-function inhibition in the group treated with 150 mg/day (60.0 ± 72.0 PRU) than in the group treated with 75 mg/day (117.0 ± 64.3 PRU; \( P = 0.004 \)). Wilcoxon rank-sum test yielded a \( P \)-value of 0.001 [median of PRU 22.0 (25th and 75th percentiles: 17.0 and 89.5) in patients treated with 150 mg/day and 130.0 (62.0 and 151.0) in patients treated with 75 mg/day].

### Discussion

This study shows that administration of 150 mg daily maintenance dose of clopidogrel results in more intense inhibition of platelet function when compared with administration of the currently recommended daily maintenance dose of 75 mg. This result was obtained with both conventional aggregometry and the VerifyNow™ P2Y12 assay. It has been shown that the results of this assay correlate with ADP-induced platelet aggregation. A large variability in platelet aggregation data was also observed in the group of patients treated with the high daily maintenance dose. The 150 mg daily maintenance dose has been used in healthy male adults in one of the dose-finding studies. In that study, subjects received 25 (\( n = 6 \)), 50 (\( n = 6 \)), 100 (\( n = 5 \)), or 150 mg (\( n = 6 \)) clopidogrel once daily, and altogether eight subjects received placebo. The treatment period was 16 days. A direct comparison with the antiplatelet effect of ticlopidine (250 mg twice daily) was missing. A dose-dependent inhibition of ADP-induced platelet aggregation was observed. However, the variability of the individual response to a 600 mg dose of clopidogrel was studied, two subacute stent thromboses occurred in those patients classified as non-responders. Matetzky et al. studied platelet function in 60 patients treated with PCI for ST-elevation myocardial infarction. During the 6 months of follow-up, eight ischaemic events occurred, of which seven occurred in the lowest quartile of response to clopidogrel.

Several prospective studies suggest that in individual patients, the degree of platelet-function inhibition correlates with the risk of thrombotic events after PCI. In a series of 105 patients, in which the variability of the individual response to a 600 mg dose of clopidogrel was studied, two subacute stent thromboses occurred in those patients classified as non-responders. Matetzky et al. studied platelet function in 60 patients treated with PCI for ST-elevation myocardial infarction. During the 6 months of follow-up, eight ischaemic events occurred, of which seven occurred in the lowest quartile of response to clopidogrel.

In another series involving 106 patients treated with PCI for an ACS, ADP- and arachidonic-acid-induced platelet aggregation was assessed at the time of the intervention. During the 1 month of follow-up, 12 ischaemic events were observed, of which nine occurred in the quartile of patients with the highest values for ADP-induced platelet aggregation. The largest prospective
study on the influence of platelet-function inhibition on the rate of adverse clinical events after PCI has been recently reported. In this study, ADP-induced platelet aggregation was assessed in 802 consecutive patients with stable coronary disease before clopidogrel loading with 600 mg and immediately before PCI. Patients were stratified in quartiles of platelet-function inhibition at the time of the intervention (level of ADP-induced platelet aggregation), and the primary endpoint was the 30-day composite of death, myocardial infarction, and target lesion revascularization (MACE), which occurred in 15 patients. Thirty-day MACE differed significantly (P = 0.03) between quartiles of platelet aggregation. Patients above the median of platelet aggregation carried a 6.5-fold risk of 30-day MACE.

The intensified clopidogrel effect of the high oral maintenance dose used in this trial has the potential to further reduce the incidence of ischaemic events after PCI. Whether the whole spectrum of patients undergoing PCI or only certain subgroups (e.g. patients with an ACS, who are known to have high baseline platelet reactivity) would benefit from the daily dose of 150 mg of clopidogrel and the duration of such a regime needs to be tested in specifically designed clinical trials. Recently, it was shown that the 150 mg daily maintenance dose is also more effective than the 75 mg daily maintenance dose in diabetic patients with a suboptimal response to clopidogrel.

An increase in bleeding complications associated with the high maintenance dose is a potential concern. Analyses of bleeding events in the recently completed Clopidogrel for High Atherothrombotic Risk and Ischaemic Stabilization, Management, and Avoidance (CHARISMA) trial suggest, though, that patients with a high atherothrombotic risk are exposed to a lower risk of severe bleeding in response to the combined therapy with clopidogrel in addition to aspirin than patients with a low atherothrombotic risk. Since it is likely that the high 150 mg daily maintenance dose will only be applied in high-risk patients, the incidence of bleeding complications with the high maintenance dose may remain acceptable.

Four limitations of this study need to be mentioned. First, baseline values of platelet function (before clopidogrel treatment) were not available. Therefore, we were not able to calculate the actual reduction of aggregation in response to clopidogrel treatment and provide the number of non- and/or poor responders according to one of the definitions used. Secondly, clopidogrel-containing capsules were used instead of commercially available tablets. Although unlikely, we cannot completely exclude that the use of the capsules had an effect on the results obtained. In an early pharmacodynamic study, platelet function assessed 2 and 5 h after administration of 400 mg of clopidogrel did not show differences between the capsule and the tablet form of the drug. Thirdly, the exclusion of patients with an ACS from this study prevents us from drawing conclusions on the effect of the 150 mg daily maintenance dose in this high-risk subset of patients. Fourth, this is a small trial that compared the effects of different clopidogrel maintenance doses on parameters of platelet function. It is far too small to comment on the safety of the higher maintenance dose. The possible clinical benefits of this regimen require confirmation in adequately powered randomized trials.

In conclusion, administration of a 150 mg daily oral maintenance dose of clopidogrel results in a more intense inhibition of ADP-induced platelet aggregation than administration of the currently recommended 75 mg daily maintenance dose.

Conflict of interest: A.K. has received lecture fees from Bristol-Myers Squibb, Lilly, and Sanofi-Aventis. VerifyNow™ P2Y12 assays were a gift from Accumetrics, Inc., San Diego, CA, USA.

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Double aortic arch and left superior vena cava persistence visualized by 16-row detector multi-slice computed tomography

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A 38-year-old woman (F.N.) suffering from dysphagia underwent a gastroscopy. An external compression of the middle portion of the oesophagus was demonstrated. The patient then underwent a 16-row detector multislice computed tomography (CT) (Lightspeed 16 pro, G.E. Medical System, Milwakee, WI, USA) with i.v. administration of iodated non-ionic contrast media (100 mL) and ECG-gated acquisition of the images. A double aortic arch was demonstrated (Panel A). The left carotid (LC) artery and the left subclavian (LS) artery originate from the left arch, whereas the right carotid (RC) artery and the right subclavian (RS) artery from the right (Panel B). This is clearly demonstrated from the endovascular view of the double arch (Panel C). Both the subclavian ostia show a diameter slightly greater than those of the carotid arteries. Along with this malformation, the persistence of the left superior vena cava draining into the coronary sinus and passing anteriorly to the left pulmonary veins was demonstrated. In Panel D, using a particular visualization of the cardiac structures (called ‘transparency’), the relationship between the superior vena cavae and cardiac structures is well valuable. Volume rendering multislice CT images seem particularly useful in complex cardiac and vascular malformations: in this case, with a single non-invasive examination, the origin of the symptoms was explained and a complete picture of the anatomical situation was obtained. This aspect is a further atout of the method when planning for a surgical approach.

Panel A. Volume rendering posterior view of the heart and the aorta. AA, ascending aorta.

Panel B. Volume rendering lateral (left) view of the aorta. Abbreviations as given in Panel A; DA, descending aorta.

Panel C. Intravascular view (‘virtual angioscopy’) of the double aortic arch. Abbreviations as given in Panel A.

Panel D. Volume rendering posterior view of the heart. Transparency reconstruction. DA, descending aorta; IVC, inferior vena cava; PV, pulmonary veins (grey); CS, coronary sinus; LVC, left superior vena cava; RA, right atrium; RVC, right superior vena cava (standard colourization).