Clopidogrel Resistance Is Associated With Increased Risk of Recurrent Atherothrombotic Events in Patients With Acute Myocardial Infarction

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Methods and Results—The antiplatelet effect of clopidogrel was studied prospectively in 60 consecutive patients who underwent primary angioplasty (percutaneous coronary intervention [PCI]) with stenting for acute ST-segment–elevation myocardial infarction (STEMI) to determine whether variability in response to clopidogrel affects clinical outcomes. Patients were stratified into 4 quartiles according to the percentage reduction of ADP-induced platelet aggregation. Although patients in the first quartile were resistant to the effects of clopidogrel (ADP-induced platelet aggregation at day 6, 103±8% of baseline), ADP-induced aggregation was reduced to 69±3%, 58±7%, and 33±12% of baseline, respectively, in patients in quartiles 2 through 4 (P<0.01 for all). In addition, epinephrine-induced platelet aggregation and platelet aggregation under flow conditions, assessed by the cone-and-plate(let) analyzer method, were reduced significantly less in the first quartile than in quartiles 2 through 4. Whereas 40% of patients in the first quartile sustained a recurrent cardiovascular event during a 6-month follow-up, only 1 patient (6.7%) in the second quartile and none in the third and fourth quartiles suffered a cardiovascular event (P=0.007).

Conclusions—Up to 25% of STEMI patients undergoing primary PCI with stenting are resistant to clopidogrel and therefore may be at increased risk for recurrent cardiovascular events. (Circulation. 2004;109:3171-3175.)

Key Words: platelets ■ myocardial infarction ■ clopidogrel

The ADP-receptor blocker clopidogrel reduces the incidence of recurrent ischemic events in patients with acute coronary syndrome (ACS)1 and after coronary stenting.2 Nevertheless, a substantial number of cardiovascular events continue to occur.1,2

Methods

Study Population

The study comprised 60 consecutive STEMI patients who were admitted within 6 hours of symptom onset. All patients underwent primary PCI with stenting, which was the standard reperfusion therapy during the study period. Ten additional consecutive patients, who underwent primary angioplasty without stenting during the study period and were not treated with clopidogrel, were also investigated. All patients were enrolled and studied prospectively.

The institutional review board approved the protocol, and patients gave written informed consent. All patients received 300 mg of chewable aspirin on admission and 200 mg/d thereafter throughout the study period. Heparin was administered during the procedure but was discouraged after the procedure. Eptifibatide was administered for a mean of 14±2 hours. Clopidogrel was administered as a loading dose of 300 mg on completion of the PCI, followed by doses of 75 mg/d for 3 months.

Blood samples for testing platelet activity were drawn in the catheterization laboratory before administration of eptifibatide at an average of 80±10 minutes after administration of chewable aspirin (baseline) and daily for 5 days thereafter.

Platelet Function Tests

Turbidimetric Platelet Aggregation

Platelet-rich plasma was prepared by centrifugation of citrated blood, and response to ADP (5 μmol/L) and epinephrine (10 μmol/L) was measured.
recorded by use of routine aggregometer (Helena Laboratory; PACKS-4).

**Cone-and-Platelet Analyzer**

This technology has been described elsewhere.8 Briefly, 200 μL of citrated blood was placed in a polystyrene well and subjected to a shear rate of 1800 s⁻¹ using a rotating conical disk for 2 minutes. The well was washed and stained by May-Gruenwald stain. Platelet adhesion was evaluated as the percentage of total area covered with platelets designated as surface coverage (%) and aggregation as the mean size of the surface-bound aggregates designated as average size (μm²) by use of an image analysis system (Galai).

**Clinical Follow-Up**

All patients were followed up via outpatient clinic visits 3 and 6 months after hospital discharge. Drug therapy compliance was assessed by telephone calls 1 month after discharge and at the first outpatient clinic visit. All medical documents were reviewed in patients who sustained recurrent cardiovascular events or underwent coronary angiography. The treating physician and the investigators who evaluated the clinical end points were blinded to the results of the platelet function activity.

**Statistical Analysis**

Platelet activity was expressed as a percentage of baseline value. Each patient served as his or her own control, and changes in platelet activity were evaluated with paired t test.

Patients were stratified into 4 quartiles based on the percentage reduction of ADP-induced platelet aggregation at day 6 compared with the baseline ADP-induced platelet aggregation (determined before clopidogrel loading). Categorical variables were presented as percentages in the 4 quartiles and tested for linear trend with Mantel-Haenszel χ² analysis. A 2-tailed Fisher’s exact test was used to compare patients in the first quartile with patients in the second through fourth quartiles. Continuous variables were presented as mean±SD. The linear trends of the variables in the 4 quartiles were compared by use of Pearson correlation. The Wilcoxon rank-sum test was used to compare patients in the first versus the second through fourth quartiles.

**Results**

Of the 60 patients enrolled, 48 (80%) were male, mean age was 58±13 years, and mean time from symptom onset to admission was 2.8±2 hours.

**Figure 1.** Changes in ADP-induced platelet aggregation (a) and aggregate size (b) after clopidogrel administration, expressed as percentage of baseline activity (*P<0.01 for changes from baseline). Changes in ADP-induced aggregation in patients receiving aspirin (ASA) (n=10) vs patients receiving clopidogrel and aspirin (n=60, §P<0.01 for comparison) (c).

**Figure 2.** Study patients (pts) were stratified into quartiles according to degree of platelet activity inhibition in response to clopidogrel treatment. Patients in 4 quartiles were compared with regard to (a) changes in ADP-induced platelet aggregation expressed as percentage of baseline activity; (b) percentage reduction in aggregate size at day 6 compared with baseline values; and (c) incidence of recurrent major adverse cardiovascular events during a 6-month follow-up.


Baseline Demographic and Clinical Characteristics of Patients

<table>
<thead>
<tr>
<th>Total Study Population (n=60)</th>
<th>1st Quartile (n=15)</th>
<th>2nd Quartile (n=15)</th>
<th>3rd Quartile (n=15)</th>
<th>4th Quartile (n=15)</th>
<th>P for Trend</th>
<th>P for 1st vs 2nd–4th Quartiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>58±13</td>
<td>60±16</td>
<td>56±15</td>
<td>55±10</td>
<td>59±14</td>
<td>0.8</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>48 (80)</td>
<td>10 (67)</td>
<td>14 (93)</td>
<td>13 (87)</td>
<td>11 (73)</td>
<td>0.8</td>
</tr>
<tr>
<td>Risk factors for CAD, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>28 (47)</td>
<td>4 (27)</td>
<td>6 (40)</td>
<td>8 (53)</td>
<td>10 (67)</td>
<td>0.023</td>
</tr>
<tr>
<td>Hypertension</td>
<td>18 (30)</td>
<td>5 (33)</td>
<td>4 (27)</td>
<td>6 (40)</td>
<td>3 (20)</td>
<td>0.6</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>16 (27)</td>
<td>3 (20)</td>
<td>5 (33)</td>
<td>6 (40)</td>
<td>2 (13)</td>
<td>0.8</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>17 (28)</td>
<td>6 (40)</td>
<td>2 (13)</td>
<td>6 (40)</td>
<td>5 (33)</td>
<td>0.9</td>
</tr>
<tr>
<td>Family history</td>
<td>8 (13)</td>
<td>2 (13)</td>
<td>3 (20)</td>
<td>1 (6.7)</td>
<td>2 (14)</td>
<td>0.8</td>
</tr>
<tr>
<td>Initial clinical characteristics, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous MI</td>
<td>8 (13)</td>
<td>2 (14)</td>
<td>2 (13)</td>
<td>2 (13)</td>
<td>2 (13)</td>
<td>1.0</td>
</tr>
<tr>
<td>Anterior infarction</td>
<td>26 (43)</td>
<td>5 (33)</td>
<td>7 (47)</td>
<td>6 (40)</td>
<td>8 (53)</td>
<td>0.3</td>
</tr>
<tr>
<td>Platelet count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>On admission, ×10^9</td>
<td>233±85</td>
<td>227±71</td>
<td>234±69</td>
<td>213±61</td>
<td>267±121</td>
<td>0.3</td>
</tr>
<tr>
<td>At day 6, ×10^9</td>
<td>241±80</td>
<td>230±86</td>
<td>238±78</td>
<td>229±47</td>
<td>264±92</td>
<td>0.3</td>
</tr>
<tr>
<td>Angiographic findings, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Multivessel CAD</td>
<td>33 (55)</td>
<td>9 (60)</td>
<td>5 (33)</td>
<td>11 (73)</td>
<td>8 (53)</td>
<td>0.7</td>
</tr>
<tr>
<td>Primary PCI success (TIMI III flow)</td>
<td>52 (87)</td>
<td>12 (80)</td>
<td>14 (93)</td>
<td>13 (87)</td>
<td>13 (87)</td>
<td>0.9</td>
</tr>
<tr>
<td>Indices of infarct size</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peak CKP, IU/L</td>
<td>1496±1234</td>
<td>1799±1485</td>
<td>1452±921</td>
<td>1361±1445</td>
<td>1417±1119</td>
<td>0.4</td>
</tr>
<tr>
<td>LVEF, %</td>
<td>43±11</td>
<td>44±11</td>
<td>42±13</td>
<td>45±11</td>
<td>43±10</td>
<td>0.9</td>
</tr>
<tr>
<td>In-hospital medical therapy, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>50 (83)</td>
<td>14 (93)</td>
<td>11 (76)</td>
<td>15 (100)</td>
<td>10 (67)</td>
<td>0.3</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>50 (83)</td>
<td>12 (80)</td>
<td>13 (87)</td>
<td>12 (80)</td>
<td>13 (87)</td>
<td>0.8</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>51 (85)</td>
<td>12 (80)</td>
<td>14 (93)</td>
<td>13 (87)</td>
<td>12 (80)</td>
<td>0.9</td>
</tr>
</tbody>
</table>

CAD indicates coronary artery disease; CKP, creatine phosphokinase; LVEF, left ventricular ejection fraction.

Antiplatelet Effect of Clopidogrel

At day 2 (18±2 hours after clopidogrel loading and 4±0.5 hours after termination of eptifibatide), both mean ADP-induced platelet aggregation and mean epinephrine-induced platelet aggregation were inhibited to 40% of baseline (P<0.01) and at day 3 to 66% (P<0.01) and 78% (P<0.01) of baseline, respectively, with no further significant changes at days 4 through 6 (Figure 1a). The final ADP aggregation (day 6) correlated negatively with the percentage inhibition of baseline ADP-induced platelet aggregation (r²=0.94, P<0.01).

The aggregate size, assessed by cone-and-platelet analysis, which reflects platelet aggregation under flow conditions, was significantly decreased to 60% of baseline value at day 2 and to ≈70% at day 6 (Figure 1b). Although the surface coverage was decreased significantly, by ≈50% (P<0.01), at day 2 when an eptifibatide effect could still be expected, clopidogrel had no significant effect on surface coverage on day 3 and thereafter (≈90% of baseline at days 3 through 6, P=0.16).

In patients who underwent angioplasty without stenting and therefore were treated with aspirin alone, no significant inhibition of ADP- and epinephrine-induced platelet aggregation at days 3 through 6 was noted compared with baseline activity, which had already been determined after aspirin loading. At each of these time points, platelet reactivity was significantly higher than in patients treated with both aspirin and clopidogrel (P<0.01, Figure 1c).

Variability in Platelet Response to Clopidogrel

When patients were stratified into 4 quartiles according to percentage reduction of ADP-induced platelet aggregation at day 6 compared with the baseline activity of each patient, significant variability was noted. Whereas patients in the first quartile were resistant to the effect of clopidogrel (ADP-induced platelet aggregation at day 6, 103±8% of baseline), ADP-induced platelet aggregation was reduced significantly in patients in the second through fourth quartiles, to 69±3%, 58±7%, and 33±12% of the respective baselines (P<0.01 for all, Figure 2a). Inhibition in platelet aggregation increased significantly in the first through fourth quartiles (P for trend <0.01). Patients in the first through fourth quartiles also exhibited increasing reduction in epinephrine-induced platelet aggregation (P for trend <0.001) and in aggregate size, reflecting aggregation under flow conditions (Figure 2b).

Clinical Outcomes

There was a gradual and significant increase in the number of cigarette smokers in the first through fourth quartiles (Table),...
but otherwise there were no significant differences in the other baseline demographic and clinical characteristics, platelet counts on admission and at day 6, indices of infarct size, and angiographic findings (Table). Similarly, there were no significant differences in the medications assigned to patients during hospitalization and at hospital discharge in all 4 quartiles (Table). Postinterventional heparin was discontinued in all patients, and none of the patients received anticoagulant therapy throughout the follow-up period.

None of the 60 patients enrolled in the study were lost to the 6-month follow-up. During this period, 2 patients developed recurrent STEMI, one of whom had 2 recurrent MIs because of stent thrombosis; 3 had recurrent ACS; and a sixth suffered an acute peripheral arterial occlusion, necessitating urgent surgery. One patient died of ischemic stroke. Thus, 7 patients had 8 recurrent cardiovascular events, 6 of which occurred during treatment with clopidogrel. Seven of the 8 events (88%) occurred in patients resistant to clopidogrel (first quartile) and 1 event (12%) in a patient from the second quartile. Accordingly, whereas 40% of patients in the first quartile (clopidogrel resistant) sustained a recurrent cardiovascular event, only 1 patient (6.7%) in the second and fourth quartiles developed an event (P for trend = 0.007, Figure 2c). In contrast, major bleeding occurred in 2 patients, both of whom were in the fourth quartile.

Patients with recurrent cardiovascular events were older (P = 0.008), had higher Killip class on admission (P = 0.004), and had a lower percentage reduction of ADP-induced platelet aggregation as early as day 3 (91 ± 21% versus 62 ± 21% percent of baseline, P < 0.001), which persisted through day 6 (90 ± 16% versus 64 ± 27%, P < 0.001, Figure 3).

**Discussion**

Although clopidogrel has been proved effective as an anti-thrombotic drug, to the best of our knowledge, this is the first study to report on its antiplatelet effect in STEMI patients and also the first to demonstrate an association between clopidogrel resistance and cardiovascular risk.

**Clopidogrel Effects in AMI Patients**

Clopidogrel yielded a 35% to 60% reduction in ex vivo platelet aggregation in normal volunteers and in patients with stable coronary disease.3-6 Clopidogrel did not confer significant inhibition of platelets, which are activated by the thrombin-related activating peptide (TRAP), suggesting that clopidogrel might be less effective in clinical situations characterized by high thrombin generation, as in ACS.5,6 Furthermore, previous studies have shown that there is a marked increase in platelet activity in patients with AMI undergoing PCI7 and that pretreatment platelet activity may negatively affect the antithrombotic effect of clopidogrel.9 These findings warrant investigation of the antiplatelet effect of clopidogrel in patients with AMI. In the present study, clopidogrel administration in STEMI patients undergoing primary PCI who were already receiving aspirin was followed by a significant inhibition of ADP-induced platelet aggregation of 35% and a significant reduction of 25% in aggregate size, indicating inhibition of aggregation under flow conditions. Clopidogrel also significantly inhibited epinephrine-induced platelet aggregation, probably by blocking the effect of secondarily released ADP.

**Clopidogrel Resistance**

Recently, laboratory documentation of aspirin resistance has been shown to predict an increased risk of cardiovascular events in patients with coronary artery disease.12 A few studies have revealed important individual heterogeneity in platelet response to clopidogrel in patients with stable coronary disease,9-11 but the clinical significance of this phenomenon has not yet been investigated. We expanded this finding by showing similar marked individual variability in response
to clopidogrel in patients with STEMI, demonstrating that such heterogeneity may possess important clinical implications. In the present study, as many as 25% of patients with STEMI were resistant to clopidogrel and subsequently were at increased risk of recurrent cardiovascular events in a 6-month follow-up.

In the present study, smoking seemed to enhance the clopidogrel antplatelet effect with a consistent gradual increase of the number of cigarette smokers from the first to the fourth quartile, opposing the possibility of this being a chance finding. Aside from smoking, no other baseline characteristic predicted the response to clopidogrel.

Clopidogrel is a prodrug, and the active metabolite formation is regulated primarily by cytochrome P450 isoenzymes 3A4 and 1A2, the latter of which is activated by the polycyclic aromatic hydrocarbons that exist in cigarette smoke. Furthermore, plasma nicotine levels similar to those reported in human smokers were shown to induce cytochrome 1A2 in rats. This possible explanation is in agreement with the findings of Lau et al, who showed that in normal volunteers and patients after coronary stenting, the individual platelet variability in response to clopidogrel correlates with cytochrome P450 metabolism activity. However, polymorphism in the ADP receptor or differences in the postreceptor signaling pathway cannot be excluded as an additional explanation for the variability in platelet responsiveness and clopidogrel resistance.

It should be mentioned that the present study is an observational one, comprising a relatively small sample size, and therefore does not allow for definitive conclusions. Nevertheless, clopidogrel resistance occurs in a significant percentage of STEMI patients and is associated with a higher risk of recurrent cardiovascular events. The question of whether increased doses of clopidogrel might overcome this resistance in nonresponsive patients warrants further investigation.

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References

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