Response to Prasugrel and Levels of Circulating Reticulated Platelets in Patients With ST-Segment Elevation Myocardial Infarction

Leor Perl, MD,* Hila Lerman-Shivek, BPPharm,† Eldad Rechavia, MD,* Muthiah Vaduganathan, MD, MPH,‡ Dorit Leshem-Lev, PhD,§ Noa Zemer-Wassercug, MD,* Oshrat Dadush, MSC,¶ Pablo Codner, MD,* Tamir Bental, MD,* Alexander Battler, MD,* Ran Kornowski, MD,* Eli I. Lev, MD*

Tel-Aviv, Jerusalem, and Petah-Tikva, Israel; and Boston, Massachusetts

Objectives
The aim of this study was to determine whether response to prasugrel is associated with the proportion of circulating reticulated platelets (RPs) in patients with ST-segment elevation myocardial infarction (STEMI).

Background
Despite better pharmacodynamic properties and clinical efficacy of prasugrel compared with clopidogrel, antiplatelet responses to prasugrel are not uniform. The mechanism of this variability in response is not clear. RPs, young hyperactive forms, are increased during situations of enhanced platelet turnover.

Methods
Patients with STEMI treated with primary percutaneous intervention (PCI) and prasugrel were tested for platelet reactivity using purinergic receptor P2Y, G-protein coupled, 12 (P2Y12) assay and multiple electrode aggregometry (MEA). RP levels were determined using flow cytometry with thiazole orange staining. Tests were performed at 2 to 4 days and 30 days post-PCI. Platelet function was compared by varying levels of RPs, analyzed as continuous (regression analysis) and categorical (tertiles) variables.

Results
Sixty-two patients were included (mean age: 57.5 ± 8 years; 21.2% women; 27.7% diabetes). At the early time point, RP levels were strongly correlated with platelet reactivity when evaluated by the P2Y12 assay (Spearman’s correlation coefficient: 0.55 for P2Y12 reaction units, –0.49 for percent inhibition) and MEA (Spearman’s: 0.50). The upper tertile of RPs displayed higher platelet reactivity compared with the middle and lower tertiles, according to P2Y12 assay and MEA. Similar results with strong correlations between RP and platelet reactivity were noted at 30 days post-PCI.

Conclusions
The proportion of circulating RPs strongly correlates with response to prasugrel in patients with STEMI treated with PCI. High levels of RPs are associated with increased platelet reactivity despite prasugrel treatment. (J Am Coll Cardiol 2014;63:513–7) © 2014 by the American College of Cardiology Foundation

Early pharmacodynamic studies of prasugrel have indicated potent and consistent platelet inhibition, with relatively low rates of high on-treatment platelet reactivity (HTPR) during treatment compared with clopidogrel (1,2). Despite these promising properties, recent studies suggest that HTPR (defined by vasodilator-stimulated phosphoprotein index) on prasugrel treatment is present in approximately one-fourth of patients with acute coronary syndromes and is associated with an increased risk of adverse cardiac events (3).

In the TRITON–TIMI 38 (Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis In Myocardial Infarction 38) pharmacodynamic substudy (4), roughly 25% to 30% of patients had HTPR 1 to 2 h after the prasugrel loading dose, which decreased to 10% at 30 days. Similarly, in recent studies in patients with ST-segment elevation myocardial infarction (STEMI), HTPR rates 2 h after prasugrel loading...
were 35% to 44% and decreased significantly thereafter (5,6). Thus, there appears to be a certain degree of clinically significant variability in response to prasugrel therapy, albeit significantly less than that observed with clopidogrel.

The causes underlying the variability in prasugrel’s antiplatelet effects are presently unclear. Genetic studies initially reported that, in contrast to clopidogrel, variations in the genes encoding cytochrome P450 (CYP) 2C19 and the adenosine triphosphate–binding cassette transporter ABCB1 (CYP2C19 and ABCB1) do not affect the response to prasugrel (7,8). However, a recent report did indicate an influence of CYP2C19*2 and *17 alleles on response to prasugrel (9). It is possible that intrinsic platelet factors, rather than factors influencing the metabolic conversion of the drug, are responsible for the variability in response. Reticulated platelets (RPs) represent younger, more hyperactive platelets, with increased volume and a greater number of dense granules than older circulating platelets (10). RPs have been shown to increase in situations of increased platelet turnover, such as acute coronary syndromes (11) and stroke (12). We have previously shown that the proportion of circulating RPs inversely correlates with clopidogrel responsiveness in patients with stable coronary artery disease (13). Accordingly, our aim was to evaluate whether response to prasugrel is associated with the proportion of RPs in the circulation in patients with STEMI undergoing primary percutaneous coronary intervention (PCI).

### Methods

**Patients.** Patients undergoing primary PCI for STEMI in the Rabin Medical Center, Petah-Tikva, Israel, were considered for study participation. Patients were excluded in cases of specific contraindications to prasugrel (age >75 years, weight <60 kg, or a history of stroke or transient ischemic attack). Other exclusion criteria included hemodynamic instability, thrombocytopenia (<100,000 cells/mm³), anemia (hemoglobin <10 g/dl), and renal failure (creatinine >2.5 mg/dl).

**Study protocol.** All patients were treated with prasugrel 60 mg and aspirin 325 mg before the cardiac catheterization and received daily prasugrel 10 mg and aspirin 100 mg thereafter. Adjunctive treatment during PCI was given.


Table 1 Baseline Characteristics of the Study Population (N = 62)*

<table>
<thead>
<tr>
<th>Demographic characteristics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.5 ± 8.0</td>
</tr>
<tr>
<td>Female</td>
<td>21.2</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.6 ± 4.8</td>
</tr>
</tbody>
</table>

Clinical characteristics

| Hyperlipidemia              | 59.1   |
| Smoking                     | 51.5   |
| Hypertension                | 43.9   |
| Diabetes mellitus           | 27.7   |
| Prior myocardial infarction | 21.2   |
| Prior CABG                  | 6.1    |

Laboratory findings

| Hemoglobin (g/dl)           | 11.9 ± 1.8 |
| Platelets (×10³ per mm³)    | 248.2 ± 71.4 |
| Mean platelet volume (fl)   | 9.3 ± 1.1  |
| Creatinine (mg/dl)          | 1.22 ± 0.6 |

Medications at discharge

| Statin                      | 93.9   |
| ACE inhibitor               | 92.4   |
| Beta-blocker                | 81.8   |
| Proton-pump inhibitor       | 53.0   |
| Peri-procedural GP IIb-IIIa inhibitor | 6.1 |

*Values are mean ± SD or %. *All patients had ST segment elevation myocardial infarction. | Current or former smokers. | ACE — angiotensin-converting enzyme; CABG — coronary artery bypass graft surgery; GP — glycoprotein.

Results

Baseline characteristics. Sixty-two patients with STEMI treated with primary PCI were initially included (mean age: 57.5 ± 8 years; 21.2% women; 27.7% diabetes). Sixteen patients were lost to follow-up and thus 46 patients were included in the follow-up analysis. Table 1 describes the baseline characteristics of the entire cohort (N = 62).

Rates of HTPR. At 2 to 4 days post-PCI, 8.1% of the patients were considered HTPR according to the P2Y12 assay and 11.3% according to MEA. At follow-up at 30 days, rates of HTPR were similar at 9.1% and 11.4%, respectively (Fig. 1).

Relationship between RPs and platelet reactivity. At 2 to 4 days post-PCI, the levels of RP were strongly correlated with platelet reactivity when evaluated by the P2Y12 assay (r = 0.55 for PRU; −0.49 for percent inhibition; p < 0.0001) and MEA (r = 0.50; p < 0.0001). Similar correlations were observed at 30 days post-PCI (r = 0.65 and −0.57 for P2Y12 assay [p < 0.0001] and 0.44 for MEA [p = 0.004]).

Figure 2 displays a representative scatterplot for the correlation between RPs and P2Y12 assay (PRU) when the data from both time points were grouped together.

In the categorical analysis (Fig. 3), at the early time point the upper tertile of RP displayed higher platelet reactivity when compared with the middle and lower tertiles, according to the P2Y12 assay (PRU and percent inhibition) and MEA (p ≤ 0.01 for all assays) (Fig. 3A). Similar trends...
were noted at the 30-day post-PCI time point, although the differences between the 3 RP tertiles in MEA values were not significant (Fig. 3B). The average level of RPs was higher at the early time point compared to the 30-day follow-up time point (18.6 ± 18% vs. 13.6 ± 10%; p = 0.04).

Discussion

Our study is the first to examine the association between response to prasugrel and the level of circulating RPs as a possible mechanism for the variability in response to the drug. It provides 2 important insights to our understanding of antiplatelet responses in patients with STEMI: 1) approximately 10% of our sample were subject to persistently on-treatment platelet reactivity despite prasugrel treatment for a few days to a month after treatment initiation; and 2) RPs, young, hyperactive platelet subpopulations, are strongly related to residual platelet reactivity in prasugrel-treated patients in a dose-dependent fashion.

Variability in prasugrel response. Our study is in concordance with prior studies that have suggested that HTPR in face of peri-procedural prasugrel therapy is not an uncommon phenomenon in acute coronary syndromes (4–6). It is noteworthy that compared to those in prior trials, rates of HTPR in our study were relatively lower (9% to 11% compared with roughly 25% in prior experiences [3–6]). These differences in HTPR rates may reflect variation in the timing of the examinations. We waited at least 48 h after prasugrel loading before platelet function measurement, whereas earlier time points (2 to 12 h post-loading dose) were utilized in previous studies (3–6). Importantly, when platelet assays were repeated at 30 days post-procedure, rates of HTPR were comparable to those reported in our investigation (4). Slight variations in the definitions of HTPR and the use of different platelet function assays across these studies may have also contributed to the differences in point estimates of HTPR.

Reticulated platelets. We previously demonstrated an association between increased platelet reactivity and the proportion of circulating RPs in healthy volunteers after aspirin administration (16) and in patients with stable coronary artery disease receiving dual antiplatelet therapy with aspirin and clopidogrel (13). This study extends these findings to a population of prasugrel-treated STEMI patients. It is believed that RPs are more physiologically active (10,17). It is possible that these cells contain mRNA (18), which enables the expression of increased amounts of adenosine diphosphate receptors and intragranular proteins. These intrinsic platelet products may explain the observed increased reactivity and reduced responsiveness to antiplatelet therapy (10,16).

Study limitations. The study findings should be considered in the context of several potential limitations. The relatively low number of enrolled patients limits causal conclusions about the role of RPs in contributing to HTPR. Unfortunately, the rate of patients lost to follow-up (~25%) at 30 days may have introduced additional bias into the study results. Furthermore, the study was not powered to assess clinical endpoints; thus, the true clinical implications of our findings are unclear. The definitions employed for prasugrel HTPR were based on thresholds validated for clopidogrel treatment (14,15). Finally, platelet function was not determined in this study using the gold standard light-transmission aggregometry. However, both MEA and P2Y12 assay perform well, and in relatively good agreement with light-transmission aggregometry, in assessing platelet reactivity during P2Y12 inhibition (14).

Conclusions

The proportion of circulating RPs strongly and inversely correlates with response to prasugrel in patients with STEMI treated with primary PCI. High levels of RPs are associated with increased platelet reactivity despite prasugrel treatment. Future larger studies assessing the clinical significance and
prognostic value of the levels of circulating RPs and residual platelet reactivity in patients with STEMI treated with prasugrel are warranted.

Reprint requests and correspondence: Dr. Eli I. Lev, Department of Cardiology, Rabin Medical Center, Cardiology, Jabotinsky Street, Petah-Tikva, Tel-Aviv University, Tel-Aviv 49100, Israel. E-mail: elil@clalit.org.il.

REFERENCES


Key Words: antiplatelet therapy • pharmacology • ST-segment elevation myocardial infarction.