Onset of optimal P2Y12-ADP receptor blockade after ticagrelor and prasugrel intake in Non-ST elevation acute coronary syndrome

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Summary
Pretreatment with a loading dose (LD) of clopidogrel or ticagrelor before percutaneous coronary intervention (PCI) in acute coronary syndrome (ACS) is supported by the guidelines, but debated following a recent meta-analysis on clopidogrel pretreatment and the ACCOAST trial. In this trial prasugrel pretreatment failed to reduce ischaemic events. The timing of optimal platelet reactivity (PR) inhibition of ticagrelor and prasugrel in non-ST-elevation ACS (NSTE ACS) is yet undetermined. In the present study, we aimed to investigate the delay required to reach optimal PR inhibition in NSTE ACS following a LD of ticagrelor or prasugrel. Consecutive patients undergoing PCI for NSTE ACS were randomised in this monocentre study. The Vasodilator-phosphoprotein index (VASP) was used to measure PR before the LD and then at 30 minutes, 1, 2, 4 and 24 hours (h) post-LD. Optimal PR inhibition was defined as a VASP<50 %. We randomised 24 patients to ticagrelor or prasugrel LD. One hour after the LD, 29 % of patients had a VASP > 50 % (ticagrelor and prasugrel: 25 vs 33 %; p=0.7). Optimal PR inhibition was obtained 2 h after the LD in both groups (12/12 with ticagrelor and 11/12 with prasugrel). At that time, the mean VASP index was 19 ± 16 % (95 %CI: 12–25). Maximal PR inhibition was reached after 4 h: 11 ± 10 % (95 %CI: 6–15). In NSTE ACS undergoing PCI a LD of ticagrelor or prasugrel given during the procedure provides optimal P2Y12-ADP receptor blockade in 2 h and maximal inhibition within 4 h.

Keywords
P2Y12-ADP receptor, vasodilator-phosphoprotein, thrombosis, pretreatment

Introduction
Optimal platelet reactivity (PR) inhibition is critical to prevent ischaemic events in acute coronary syndrome (ACS) and in particular for patients undergoing percutaneous coronary intervention (PCI). The addition of a P2Y12-ADP receptor antagonist to aspirin led to a dramatic decrease in major adverse cardiovascular events and early stent thrombosis through the inhibition of thrombus formation and growth (1, 2). Clopidogrel has a delayed onset of action and a mild biological efficacy (3, 4). Therefore the use of a loading dose (LD) as a pretreatment was proposed in order to reach an optimal PR inhibition as quickly as possible. Pretreatment with clopidogrel was further clinically validated in the CURE and CREDO trials where it resulted in a reduction in ischaemic recurrences (2, 5). Accordingly, the guidelines supported pretreatment in non-ST-elevation acute coronary syndromes (NSTE ACS) (6). In these trials the delay between admission and PCI was long and does not reflect actual practices. In contemporary practice most patients undergo PCI within 24 hours (h) following admission. In addition, in the recent years the advent of prasugrel and ticagrelor has changed the landscape of P2Y12-ADP receptor blockade. These drugs have a faster onset of action and higher potency. Ticagrelor and prasugrel were subsequently shown to be superior to clopidogrel to prevent ischaemic recurrences in ACS (7, 8).

A recent meta analysis discuss the potential benefit of clopidogrel pre treatment (9). In addition, the recently published ACCOAST trial failed to show a benefit of pretreatment compared to per procedural loading with prasugrel in ACS undergoing early PCI (10). In addition the recent ATLANTIC study investigating pretreatment in ST elevation MI was also negative (11). Therefore the use of pretreatment with the new P2Y12-ADP receptor antagonists is debated. Of importance, the reasons for this failure are yet unknown. In STEMI 2 previous studies demonstrated that despite their improved pharmacodynamic and kinetic compared to clopidogrel, new P2Y12-ADP receptor antagonists have a delayed onset of action in ST-elevation myocardial infarction (12, 13). To date no
study has specifically assessed the time required in non ST-elevation ACS for these new drugs to reach an optimal PR inhibition. This data is critical in the current debate over pre-treatment and could explain the lack of benefit in the ACCOAST trial. Accordingly, the present study investigated the timing of onset of optimal P2Y12-ADP receptor blockade following intake of a LD of ticagrelor or prasugrel in NSTE ACS undergoing PCI.

**Methods**

A prospective monocentre, open-label randomised study was performed between November 2014 and January 2015. Patients between 18 and 75 years old undergoing PCI for an intermediate or high-risk NSTE-ACS and agreeing to participate in the study were eligible. The present study protocol was approved by the institution’s human research committee and conforms to the Declaration of Helsinki. All patients gave informed consent.

Exclusion criteria included ST-elevation myocardial infarction, NSTE-ACS medically managed or intended for surgery, cardiogenic shock, cardiac arrest, contra-indication to antiplatelet therapy, treatment with a P2Y12-ADP antagonist <1 month, a platelet count <100 G/l, history of bleeding diathesis, history of haemorrhagic stroke, stroke, recent surgery (<1 month), age ≥75 years old, weight <60 kg, haemodialysis, use of medication with known interference with ticagrelor or prasugrel and bradycardia.

PCI was performed using the radial route in all cases. All patients received a bolus of heparin (70 UI/kg) during the procedure followed by ACT-adjusted additional bolus. Drug-eluting stents were used in all patients. No patients received GP IIb/IIIa inhibitors.

**Antiplatelet therapy**

Patients in both groups received a 150 mg intra-venous LD of aspirin followed by 75 mg per os daily. Patients were randomised 1:1 to the prasugrel or the ticagrelor group after coronary angiography and once PCI was chosen as the therapeutic strategy. In the prasugrel group a 60 mg LD was given followed by a 10 mg daily dose. In the ticagrelor group patients received a 180 mg LD followed by 90 mg twich daily (6).

**Timing of platelet reactivity measurement**

PR was measured before the LD and then at 30 minutes (min), 1, 2, 4 and 24 h post-loading in order to determine the timing of optimal PR inhibition.

**Platelet reactivity measurements**

Platelet reactivity was measured using the VASP index. Blood samples for VASP index analysis were drawn by anatraumatic venipuncture of the antecubital vein. The initial blood drawn was discarded to avoid measuring platelet activation induced by needle puncture. Blood was collected into a Vacutainer containing 3.8% trisodium citrate and filled to capacity. The Vacutainer was inverted 3-5 times for gentle mixing and sent immediately to the haemostasis laboratory. VASP index phosphorylation analysis was performed within 24 h of blood collection by an experienced investigator using the CY-QUANT VASP/P2Y12 enzyme-linked immunoassortment assay (Biocytex, Marseille, France) (14, 15). Briefly, after a first step of parallel whole blood sample activation with PGE1 and PGE1+ADP platelets from the sample are lysed, allowing released VASP to be captured by an anti-human VASP antibody which is coated in the microtitre plate. Then, a peroxidase-coupled anti-human VASP-P antibody binds to phosphorylated serine 239 antigenic determinant of VASP. The bound enzyme peroxidase is then revealed by its activity on TMB substrate over a predetermined time. After stopping the reaction, absorbance at 450 nm is directly related to the concentration of VASP-P contained in the sample. The VASP index was calculated using optical density (OD 450 nm) of samples incubated with PGE1 or PGE1

<table>
<thead>
<tr>
<th>n (%)</th>
<th>Ticagrelor group (n=12)</th>
<th>Prasugrel group (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Mean ±SD)</td>
<td>63 ±11</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>Males</td>
<td>9 (75)</td>
<td>10 (83)</td>
</tr>
<tr>
<td>BMI (kg/m²)(Mean ±SD)</td>
<td>29 ± 4.3</td>
<td>29.1 ± 8.9</td>
</tr>
<tr>
<td>Cardiovascular risk factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>6 (50)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Hypercholesterolaemia</td>
<td>6 (50)</td>
<td>7 (58)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>8(67)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5 (42)</td>
<td>4 (33)</td>
</tr>
<tr>
<td>Family history of CAD</td>
<td>1 (8)</td>
<td>2 (16)</td>
</tr>
<tr>
<td>Clinical setting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSTEMI</td>
<td>6 (50)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Unstable angina</td>
<td>6 (50)</td>
<td>6 (50)</td>
</tr>
<tr>
<td>Medication on admission</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>5 (42)</td>
<td>3 (25)</td>
</tr>
<tr>
<td>BetaBlocker</td>
<td>3 (25)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statin</td>
<td>5 (42)</td>
<td>4 (34)</td>
</tr>
<tr>
<td>Insulin</td>
<td>1 (8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Oral anti-diabetics</td>
<td>3 (25)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Biology (Mean ±SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leucocytes (x10⁹/l)</td>
<td>9.78 ± 3.92</td>
<td>8.92 ± 2.45</td>
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<tr>
<td>Haemoglobin (g/dl)</td>
<td>13.7 ± 2.23</td>
<td>14.8 ± 2.45</td>
</tr>
<tr>
<td>Platelets (x10⁹/l)</td>
<td>237 ± 65</td>
<td>242 ± 61</td>
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<tr>
<td>Fibrinogen (g/l)</td>
<td>3.67 ± 0.75</td>
<td>3.89 ± 0.85</td>
</tr>
<tr>
<td>Creatinin (µmol/l)</td>
<td>95 ± 32</td>
<td>78 ± 21</td>
</tr>
</tbody>
</table>

BMI : Body Mass Index; CAD : Coronary Artery Disease; NSTEMI : Non ST-Elevation Myocardial Infarction.
and ADP according to the formula: \[ VASP = \left( \frac{OD450nm_{(PGE1)} - OD450nm_{(PGE1+ADP)}}{OD450nm_{(PGE1)}} \right) \times 100. \] Optimal PR inhibition was defined as a VASP index < 50% according to the consensus document on the definition of high on-treatment platelet reactivity (3). Maximal PR was defined as the maximal PRI reached during the study.

Statistical analysis
Analyses were performed with the Graphpad Prism software v5.0 for windows (Graphpad Software Inc., San Diego, CA, USA). All tests were two-sided and considered significant if \( p < 0.05 \). Categorical data are expressed as counts (%) and were compared using \( \chi^2 \) or Fisher's exact tests. Continuous variables are expressed as mean ± standard deviation (SD) and were compared using the Mann-Whitney test.

Results
Baseline characteristics
We included 24 patients divided in the ticagrelor (n=12) and the prasugrel (n=12) group. The baseline characteristics of the study population are depicted in Table 1.

The two groups were similar regarding the main clinical, angiographic and biological parameters. It was the case of mostly young patients: 58.5 years old (95%: 54–63). Half of patients were admitted for NSTEMI. Diabetes was present in 37.5% of patients. No patients received morphine during hospital stay.

Timing of onset of action
The value of PR, assessed by the VASP, at each time point for the overall population and for each group is depicted in Figure 1A and B, respectively. At baseline, all patients had a PR above 70% (90 ± 7%; 95% confidence interval [CI]: 87–93) which is the reference value in healthy volunteers. The mean VASP was significantly decreased at 30 min compared to baseline (\( p < 0.001 \)) but remained above the 50% cut-off value of VASP (70 ± 26%; 95% CI: 59–82). Only three patients in each group (25%) had a PR below 50% at that time.

One hour following the LD of either prasugrel or ticagrelor the mean PR was 36 ± 29% (95% CI: 24–49). However 29% of patients still had a PR > 50% (3/12 in the ticagrelor group and 4/12 in the prasugrel group; \( p = 0.7 \)).

Optimal PR inhibition was achieved 2 h after the LD of either ticagrelor or prasugrel (12/12 in the ticagrelor group and 11/12 in the prasugrel group) (mean PR: 19 ± 16%; 95% CI: 12–25) in all patients but one. At 4 h maximal PR was reached and remained stable at 24 h (11 ± 10%; 95% CI: 6–15).

Comparison between ticagrelor and prasugrel
In the present study at all time points PR inhibition achieved by ticagrelor and prasugrel were similar. Both the potency and timing of onset of action were identical between the two drugs (\( p = \text{ns} \)) (Figure 2). In the prasugrel group, one patient had a PR > 50% at all time points.

Discussion
The present study demonstrates that in NSTEMI undergoing PCI, optimal PR inhibition is reached 2 h after a loading dose of either ticagrelor or prasugrel. In addition, maximal PR inhibition is obtained within 4 h. Interestingly, both drugs had a similar onset of action. The present findings suggest that P2Y12-ADP receptor blockade is quickly reached after the LD but is not optimal during the intervention.

These findings provide critical data for interventionalists regarding the use of pretreatment and the pharmacological environment of PCI. The present study specifically addresses the timing of optimal PR inhibition in NSTEMI ACS following a LD of ticagrelor or prasugrel. In the PRINCIPLE TIMI 44 and in the ONSET / OFFSET studies, the timing of action of the new P2Y12-ADP antagonists was inferior to 1 h (16, 17). However, these studies were performed in stable coronary artery disease patients. ACS patients have a heightened platelet activity and exhibit a lower efficacy of antiplatelet agents compared to stable patients (12, 18). Subsequently despite the high biological efficacy of prasugrel and ticagrelor, previous studies demonstrated that in STEMI the biological efficacy of both drugs was delayed until at least 4 h after the LD (12, 13). The present study demonstrates that although longer than in stable patients, ticagrelor and prasugrel provided an optimal PR (i.e. VASP < 50%) 2 h after the oral LD which is faster than in STEMI. The practice of early PCI and the quick onset of action of these drugs reduce the time window when ischaemic recurrences could occur. The potential advantage of pretreatment is therefore limited. Accordingly, in the ACCOAST trial prasugrel pretreatment did not reduce the rate of ischaemic event compared to per-procedural loading. In this trial PCI was performed early after admission (10). Thus the quick onset of action of prasugrel and ticagrelor in the present study may explain the lack of clinical efficacy of pretreatment.

Figure 1: Time dependence of P2Y12-ADP receptor blockade assessed by the VASP index in the overall population (n=24) (A) and in the ticagrelor (n=12) and the prasugrel group (n=12) (B).
In the present mechanistic study, we further evidence that ticagrelor had a similar delay of action compared to prasugrel. This finding is of interest and suggests that factors related to intestinal absorption or intrinsic platelet activity are responsible for the delay onset of action since prasugrel unlike ticagrelor is a pro-drug and therefore requires bio-transformation to be active (3). However, our study has a limited sample size and therefore may not be powered to show differences between the two drugs. The mechanisms of the delayed onset of action in NSTE ACS for the new P2Y12 antagonists compared to what was observed in stable patients may be related to the heightened platelet activity observed in this clinical setting and the high platelet turn over (18–21). On the

![Figure 2: Comparison of platelet reactivity assessed by the VASP index at each time points showing no difference between the two groups. A) Baseline; B) at 30 min after the loading dose; C) at 1 h after the loading dose; D) at 2 h after the loading dose; E) at 4 h after the loading dose; F) at 24 h after the loading dose.](image-url)
other hand, the onset of action in NSTE ACS appears to be reduced compared to STEMI. This is likely related to the fact that factors implicated in the delayed onset of action in STEMI are not present in NSTE ACS including haemodynamic compromise, systemic vasoconstriction, adrenergic activation and splanchnic hypoperfusion which are responsible for delayed drug absorption (18). In addition, co-medication with morphine, which delayed the biological efficacy of P2Y12-ADP antagonists, is uncommon (22). Finally the thrombus burden in NSTE ACS is reduced compared to STEMI (20).

From our results, it must be underlined that per procedural intake of the LD does not provide an optimal PR inhibition during the interventional procedure which suggests that if a thrombotic complication occurs, an intravenous drug such as cangrelor or GP IIb/IIIa inhibitors should be used to prevent further thrombus formation during the first 2 h (6).

Limitations

In the present study only one platelet assay was used. However, the VASP index assay is well validated to assess P2Y12-ADP receptor blockade and was largely used in the literature. This study aimed to assess the timing of onset of optimal PR inhibition. Therefore no power calculation was made and we do not have the power to compare the biological efficacy in steady state of prasugrel and ticagrelor due to the small sample size. This was investigated by previous studies (23). The definition of optimal platelet reactivity is based on the consensus definition of ‘high on-treatment platelet reactivity’, but is not a measure of the clinical risk benefit ratio.

Conclusion

In the present study we demonstrate that in non ST-elevation ACS undergoing PCI a loading dose of ticagrelor or prasugrel given during the procedure provides optimal P2Y12-ADP receptor blockade in 2 h and maximal inhibition within 4 h. Both drugs had a similar timing of onset of action. Such findings support the lack of potential benefit of preloading with these new agents given their quick efficacy in this clinical setting.

Acknowledgements

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Conflicts of interest

Laurent Bonello received lecture fees from Sanoﬁ, Eli Lilly, AstraZeneca and Medicine company and research grant from AstraZeneca. Marc Laine received lecture fee from AstraZeneca. Franck Paganelli received lecture fees from Sanoﬁ, Eli Lilly, and AstraZeneca and research grant from AstraZeneca. None of the authors have stock ownership or other equity interests or patent licensing arrangements with AstraZeneca or Eli Lilly.

References


What is known about this topic?

• Guidelines recommend a loading dose of ticagrelor or clopidogrel at the time of diagnosis in non ST-elevation acute coronary syndrome.
• The ACCOAST trial failed to show a benefit of a loading dose of prasugrel in this clinical setting.
• The pharmacodynamic and kinetic properties of ticagrelor and prasugrel are unknown in this clinical setting.

What does this paper add?

• The onset of optimal platelet reactivity inhibition in non ST-elevation acute coronary syndrome following a loading dose of ticagrelor or prasugrel is 2 h.
• The loading dose of prasugrel and ticagrelor have a similar pharmacodynamic and kinetic properties.
• Our findings support the lack of potential benefit of a loading dose of the new P2Y12-ADP antagonists in this clinical setting in relation to their quick onset of action.

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