Original Article
Ticagrelor enhances adenosine-mediated antiplatelet effect in acute coronary syndrome patients—a prospective cohort study

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Abstract: Background: Ticagrelor could exert a potential higher antiplatelet effect than clopidogrel and inhibit cellular uptake of adenosine, which was associated with several cardiovascular effects. We aimed to explore the association of plasma adenosine concentration (APC) and antiplatelet effect with clopidogrel or ticagrelor in acute coronary syndrome (ACS) patients receiving dual antiplatelet therapy (DAPT). Methods: In this prospective, observational, and single-center cohort study, we enrolled 71 patients treated with ticagrelor and 85 with clopidogrel. We monitored the inhibition platelet aggregation (IPA) and assessed the adenosine and cyclic adenosine monophosphate (cAMP) plasma concentration. Clinical data and cardiovascular events of 30 days were also collected to investigate the differences between the groups and hazard ratios during prognosis. Results: The IPA of ticagrelor was significantly higher than clopidogrel (88.30 ± 13.06% vs. 60.02 ± 28.38%, P=0.001). The APC was significantly higher in ticagrelor therapy than clopidogrel therapy (2.88 ± 0.62 µmol/L vs. 2.56 ± 0.40 µmol/L, P=0.019). The cAMP plasma concentration was also significantly higher in ticagrelor therapy than clopidogrel therapy (21.91 ± 9.15 pmol/ml vs. 18.43 ± 9.02 pmol/ml, P=0.013). The plasma levels of adenosine and cAMP were positively correlated with ADP-induced IPA (r=0.68, P<0.001; r=0.30, P=0.002, respectively). The multivariate analysis adjusted for the dyspnea revealed that the treatment with ticagrelor was independently associated with this adverse drug reaction (odds ratio (OR), 5.295; 95% confidence interval (CI), 1.429-19.617). Conclusion: Results from this study indicated that ticagrelor could increase adenosine and cAMP plasma concentration and had a potential antiplatelet effect in patients with ACS that might be adenosine-mediated.

Keywords: Ticagrelor, acute coronary syndrome, adenosine, cyclic adenosine monophosphate

Introduction

Ticagrelor is an oral P2Y12 receptor antagonist [1] that is recommended for the prevention of clinical thrombotic events in patients with acute coronary syndrome (ACS), according to the 2014 ESC/EACTS guidelines [2] on myocardial revascularization. Ticagrelor is a more potential antiplatelet drug than clopidogrel, especially for patients with poor CYP2C19 metabolism and low inhibitor platelet aggregation [3]. In PLATO study [4], ticagrelor could reduce the major adverse cardiac and cerebrovascular events (MACCE) compared to the standard treatment with clopidogrel. Also, a greater incidence of dyspnea was observed with the medication of ticagrelor [5]. These findings led to the hypothesis that the pleiotropic effect of ticagrelor was mediated through a novel non-platelet directed mechanisms of action.

A number of studies concluded that ticagrelor might inhibit the cellular uptake of adenosine through the equilibrative nucleoside transporter-1 (ENT-1) [6], consequently leading to a superior effect on coronary blood flow and platelet aggregation as compared to clopidogrel in animal models [7]. Adenosine, a regulatory metabolite, is an important mediator of inhibition of platelet activation [8]. The extracellular adenosine activates the platelet A2AR [9], which in turn stimulates the production by adenylyl cyclase of cAMP [10, 11] representing a dual mode of platelet aggregation inhibition by ticagrelor.
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Thus, the higher potency and persistent effect on antiplatelet aggregation might be attributed to the possible adenosine-like actions of ticagrelor [12]. Clopidogrel has been the most prescribed medication for myocardial revascularization for ACS patients but, it does not have an inhibitory effect on adenosine cellular uptake.

Most of the current evidence on the effect of ticagrelor on adenosine metabolism is derived from in vitro studies or from studies in healthy volunteers wherein the adenosine metabolism could not reflect the clinical effect. Therefore, in the current study, we aimed to test this hypothesis in ACS patients in vivo. Here we carried out a prospective, observational, and single-center cohort study in 156 patients with ACS, who never received ticagrelor and clopidogrel therapy previously. The purpose of our study was to investigate the relevance of ticagrelor induced increasing adenosine and cAMP plasma concentration with the inhibition of platelet aggregation (IPA).

Materials and methods

We performed a prospective, observational, and single-center cohort study enrolling patients with non-ST-segment elevation ACS. The protocol was approved by the Ethical Committee of Zhongshan Hospital, Fudan University. It was conducted in accordance with Good Clinical Practice and compliance with the Helsinki Declaration. Informed consent was obtained from all the patients, before undergoing any study procedure.

Study design

From January 2016 to March 2016, a total of 156 Chinese patients with non-ST-segment elevation ACS undergoing PCI were enrolled in this study. All the patients were allocated to ticagrelor or clopidogrel according to the clinician practice or evidence-based medicine. Blood samples were collected before coronary angiography and 48 h after a P2Y\textsubscript{12}–ADP receptor antagonist loading dose.

Each subject underwent a characterization including the history of smoking or alcohol consumption, comorbidity disease, hemoglobin, hematocrit, platelet count, alanine aminotransferase (ALT), estimated glomerular filtration rate (eGFR), left ventricular ejection fraction (LVEF), and the number of stents. We monitored the ticagrelor induced adverse drug reactions of dyspnea and atrial ventricular block for each patient according to the PLATO clinical trial and C-reactive protein (CRP). We determined the IPA by thromboelastogram (TEG). The comparison of the MACCE in 30 days between the two groups was conducted. The plasma concentrations of adenosine and cAMP were detected by fluorescent probe adenosine assay kit for each.

The study was carried out at Cardiovascular Division of Zhongshan Hospital, Fudan University, Shanghai, China. The ACS was diagnosed according to the European Society of Cardiology (ESC) criteria [2]. Patients eligible for inclusion were presented with a history of chest pain lasting for more than 20 min, unresponsive to nitroglycerine. The ECG showed an ST segment and T wave alterations. The cardiac markers showed a typical rise. All the patients were treated with primary percutaneous coronary intervention for reperfusion and drug eluting stents were implanted. Patients never medicated with P2Y\textsubscript{12} receptor antagonist were included. The exclusion criteria were age less than 18 years, allergies or contraindications to either ticagrelor or clopidogrel, a history of cerebral hemorrhage, increased risk of bleeding and hematologic disorder, severe liver and kidney insufficiency, acute and chronic infection, platelet count <100×10\textsuperscript{9}, requiring coronary artery bypass graft (CABG) surgery after coronary angiography.

For antiplatelet therapy, a loading dose of 180 mg ticagrelor followed by a daily regimen of 90 mg twice was administered in the ticagrelor group and that of 300 mg clopidogrel by a daily regimen of 75 mg once in the clopidogrel group. In addition to the P2Y\textsubscript{12} receptor antagonist antiplatelet treatment, all the subjects received aspirin for antithrombotic therapy, low molecular weight heparin for anticoagulation, statins for stable plaque, angiotensin converting enzyme inhibitors (ACEI) or angiotensin receptor blocker (ARB) for ventricular remodeling, beta-block for reducing myocardial oxygen consumption, and with proton pump inhibitors (PPI) for gastrointestinal discomfort.

Evaluation of platelet function

At 48 h post the clopidogrel or ticagrelor loading dose, the magnitude of platelet reactivity
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was assessed by Thrombelastograph Hemostasis Analyzer (Haemoscope Corp, Niles, IL, USA). The contribution of P2Y<sub>12</sub> receptor pathway to the clot formation could be measured by the addition of the agonist of adenosine diphosphate (ADP). The maximum amplitude of TEG scanning demonstrated the strength of the blood clot that was being formed. The platelet and fibrosis are the major contributors to the process. Thrombin that induced clot strength (MA<sub>Thrombin</sub>) reflects the patient’s maximal potential platelet reactivity [13]. MA<sub>Fibrin</sub> reflects the contribution of fibrin alone to the clot strength, whereas MA<sub>ADP</sub> represents the contribution of platelets not inhibited by clopidogrel or ticagrelor. Platelet aggregation in response to ADP was calculated with software by the formula.

\[
\text{ADP-induced platelet aggregation (\%)} = \left( \frac{\text{MA}_{\text{ADP}} - \text{MA}_{\text{Fibrin}}}{\text{MA}_{\text{Thrombin}} - \text{MA}_{\text{Fibrin}}} \right) \times 100\%.
\]

Laboratory parameters

Fasting blood samples were withdrawn under standardized conditions and stored at -80°C until further analysis. Biochemical and routine blood tests were conducted by the Department of Laboratory Medicine of Zhongshan Hospital. Hs-CRP was measured using a particle-enhanced immunoturbidimetric assay (DiaSys Diagnostic Systems, Shanghai, China) on the HI-7600-120 Analyser (HITA). APC was measured by adenosine deaminase followed by a multi-step enzymatic approach resulting in the generation of an intermediate that reacts with the adenosine probe forming a fluorescent product. The fluorescent product (Bio Vision, CA, USA) was measured at Ex/Em=535/587 nm within a detection range of 2-80 pmol. The cAMP assay (R&D system, Minneapolis, USA) was based on the competitive binding tech-
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Follow-up

All patients included in the study were followed up for 30 days from the discharge day. Major adverse cardiac and cerebrovascular events including cardiac death, occurrence of new Q myocardial infarction, major stroke, the need for target lesion revascularization, CABG performed after the emergency procedure, recurrent hospitalization for angina or congestive heart failure were recorded. All participants were followed by their medical records.

Statistical analysis

Comparisons of continuous variables between the two groups were performed by Student’s t-test or Wilcoxon two sample test. Paired Student t-test or Wilcoxon sign sum test were used for comparison of intra-group continuous variables at before and after treatment. Repeated measures analysis of variance was performed to determine the main effect of repeated measures data between two groups. Comparisons of categorical variables were assessed by Chi-square or Fisher’s exact test. Pearson’s r coefficient of correlation was used for correlation studies. Binary multivariate logistic regression analysis was performed to assess the adjusted hazard of adverse events. Data were analyzed using IBM SPSS Statistics 19.0 software, all statistical analysis was two-tailed test, P value <0.05 was considered statistical significance.

Results

Clinical characteristics

156 patients with non-ST-segment elevation ACS were enrolled in this study including 71 in ticagrelor group and 85 in clopidogrel group. The demographic and clinical characteristics of these study participants in the two groups were presented in Table 1. No statistical differences were observed in age, sex, body mass index (BMI), hypertension, diabetes, stroke, liver and kidney function, hemoglobin, hematocrit, platelet (PLT), left ventricular ejection fraction (LVEF), the number of stents, and medication between the two groups.

The ADP-induced IPA, CRP, hospital stays and costs

Compared to the clopidogrel treatment, ticagrelor had a putative effect on IPA. The inhibitor of ADP-induced platelet aggregation was markedly higher with ticagrelor treatment than the clopidogrel treatment (88.3 ± 13.06% vs. 60.02 ± 28.38%, P<0.001). Regarding the CRP levels, we observed no difference between patients treated with ticagrelor and clopidogrel (32.83 ± 39.26 vs. 19.5 ± 15.52, P=0.234). Also, there is no significant difference in the hospital stays and costs of participants of two groups (Table 2).

The changes of the adenosine and cAMP levels

The plasma levels of adenosine and cAMP were similar between the patients with ticagrelor and clopidogrel treatment during admission (P=0.895 and P=0.351, respectively).
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48 h after receiving the loading dose of P2Y12 antagonists, APC tended to increase after ticagrelor treatment (P=0.042). Contrastingly, APC with clopidogrel treatment decreased after receiving the loading dose (P=0.278). Furthermore, the adenosine level was higher in the ticagrelor treatment than the clopidogrel treatment (P=0.001). The cAMP plasma concentration tended to increase after ticagrelor and clopidogrel treatment (P<0.001 for both). The cAMP plasma concentration significantly increased in patients receiving ticagrelor than those with clopidogrel (P=0.013) (Table 3).

Laboratory parameters and IPA

48 h after receiving the clopidogrel or ticagrelor loading dose, we assessed the inhibitory effect on platelet aggregation by TEG and monitored the plasma concentration of adenosine and cAMP. The APC and cAMP plasma concentration were positively correlated with IPA in ACS patients (r=0.68, P<0.001; r=0.30, P=0.002, respectively) (Figure 1).

Adverse drug reaction and re-hospitalization

Significant differences existed in adverse drug reactions, which indicated that ticagrelor could increase the incidence of dyspnea compared with clopidogrel (P=0.028). However, there was no significance observed in the two groups with atrioventricular block and bleeding. All the study subjects were successfully followed up for 30 days from the discharge. Four patients in the ticagrelor group and thirteen from clopido-

Table 3. APC and cAMP of participants at before and after treatment (mean ± sd.)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Time</th>
<th>Clopidogrel (n=85)</th>
<th>Ticagrelor (n=71)</th>
<th>Statistic value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC (μmol/L)</td>
<td>Pre-treatment</td>
<td>2.65 ± 0.51</td>
<td>2.69 ± 0.60</td>
<td>0.132</td>
<td>0.895a</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>2.56 ± 0.40</td>
<td>2.88 ± 0.62</td>
<td>3.354</td>
<td>0.001a</td>
</tr>
<tr>
<td></td>
<td>Statistic value</td>
<td>-1.092</td>
<td>2.068</td>
<td>8.590</td>
<td>0.004c</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>0.278a</td>
<td>0.042a</td>
<td>0.840</td>
<td>0.019d</td>
</tr>
<tr>
<td>cAMP (pmol/mL)</td>
<td>Pre-treatment</td>
<td>10.08 ± 5.10</td>
<td>9.55 ± 4.69</td>
<td>-2.036</td>
<td>0.351a</td>
</tr>
<tr>
<td></td>
<td>Post-treatment</td>
<td>18.43 ± 9.02</td>
<td>21.91 ± 9.15</td>
<td>2.477</td>
<td>0.013a</td>
</tr>
<tr>
<td></td>
<td>Statistic value</td>
<td>7.013</td>
<td>9.408</td>
<td>2.690</td>
<td>0.103c</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt;0.001a</td>
<td>&lt;0.001a</td>
<td>138.35</td>
<td>&lt;0.001d</td>
</tr>
</tbody>
</table>

Note: a, P value of intra-group comparison before and after treatment. b, P value of inter-group comparison. c, P value of inter-group effect using repeated measures analysis of variance; d, P value of time effect using repeated measures analysis of variance.

Figure 1. The positive correlation between laboratory parameters and IPA in ACS patients by adenosine plasma concentration (A) and cAMP plasma concentration (B).
### Table 4. Adverse drug reactions and re-hospitalization of the two groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Clopidogrel (n=85)</th>
<th>Ticagrelor (n=71)</th>
<th>Statistic value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnea, n (%)</td>
<td>5 (5.88)</td>
<td>12 (16.90)</td>
<td>4.837</td>
<td>0.028*</td>
</tr>
<tr>
<td>Atrioventricular block, n (%)</td>
<td>4 (4.71)</td>
<td>5 (7.04)</td>
<td>0.077</td>
<td>0.781</td>
</tr>
<tr>
<td>Bleeding, n (%)</td>
<td>2 (2.35)</td>
<td>3 (4.23)</td>
<td>0.041</td>
<td>0.838</td>
</tr>
<tr>
<td>Re-hospitalized, n (%)</td>
<td>13 (15.29)</td>
<td>4 (5.63)</td>
<td>3.718</td>
<td>0.054</td>
</tr>
</tbody>
</table>

*The crude RR for Ticagrelor vs. Clopidogrel was 2.873 (95% CI, 1.062-7.768).

### Table 5. The adjusted hazard of dyspnea for Ticagrelor vs. Clopidogrel (multivariate logistic regression)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Beta value</th>
<th>Standard error</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.5748</td>
<td>1.1344</td>
<td>-</td>
<td>0.6124</td>
</tr>
<tr>
<td>Age (years), &lt;60 vs. ≥60</td>
<td>0.3559</td>
<td>0.3233</td>
<td>2.038 (0.574-7.238)</td>
<td>0.2710</td>
</tr>
<tr>
<td>Ticagrelor vs. Clopidogrel</td>
<td>0.8334</td>
<td>0.3341</td>
<td>5.295 (1.429-19.617)</td>
<td>0.0126</td>
</tr>
</tbody>
</table>

Note: the dependent variable of multivariate logistic regression was dyspnea, independent variables including age and treatment.

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The multivariate analysis adjusted for the dyspnea revealed that the treatment with ticagrelor was independently associated with this adverse drug reaction (OR=5.295; 95% CI, 1.429-19.617) (Table 5).

### Discussion

Platelets express a broad range of receptors that play essential roles in thrombus formation, which might be a high risk of ACS [14]. Among these, the P2Y\(_{12}\) receptor, a member of the G protein-coupled receptor family, has attracted a significant amount of attention [12, 15]. Stimulation of the P2Y\(_{12}\) receptor by ADP results in the activation of various signaling pathways involved in the amplification of platelet activation and aggregation [16, 17]. Clopidogrel that is metabolized by CYP2C19 in the liver to form an active metabolite is the most widely used P2Y\(_{12}\) receptor antagonist with high efficacy for the ACS patients [18-20]. Regarding antiplatelet effect, ticagrelor is more potent than clopidogrel and produces a rapid and robust inhibition of platelet aggregation. Moreover, ticagrelor could inhibit ENT-1 to exert cardioprotective effects by increasing the plasma concentration of adenosine in healthy volunteers [21]. Antiplateleting is a biological effect of adenosine carried out by the mechanism of inhibiting the platelet activation mainly via A\(_{1}\)R but also via A\(_{2A}\)R [8]. In agreement with the previous evidence [6], our study demonstrated that ticagrelor was associated with an increased adenosine and cAMP plasma concentration with a potential antiplatelet effect on ACS patients. We further identified that the adenosine and cAMP were positively correlated with the ADP-induced IPA. These observations suggested that the potential antiplatelet effect of ticagrelor might be mediated through the increasing adenosine and cAMP plasma concentrations. Also, the accumulation of adenosine in the blood would eventually lead to dyspnea in ticagrelor-treated patients to affect the C fibers of the vagus nerve via the activation of adenosine A\(_{1}\) and A\(_{2}\) receptors [8].

We found that 48 h after receiving the loading dose of P2Y\(_{12}\) antagonists, the inhibition of ADP-induced platelet aggregation was 88.3% for ticagrelor versus 60.0% for clopidogrel (P<0.001), respectively. The results of the study documented that ticagrelor was inferior to clopidogrel regarding the antiplatelet effect as a consequence of the loading dose. The RESPOND clinical trial [22] showed that ticagrelor could improve the antiplatelet effect for nonresponsiveness to clopidogrel. Moreover, the ONSET/OFFSET clinical trial [23] demonstrated that Ticagrelor achieved greater IPA rapidly than the high-loading-dose clopidogrel. 24 h post the last dose, the mean IPA was 58% for ticagrelor vs. 52% for clopidogrel. Our results were thus consistent with the previous studies. Ticagrelor is an ADP receptor antagonist that binds reversibly in a non-competitive manner to the P2Y\(_{12}\) receptor at distinct sites on ADP and does not require metabolic activation to achieve its antiplatelet activity [3, 24].

By monitoring the plasma adenosine concentration, we also observed that the potential
antiplatelet property of ticagrelor was related to the inhibition of adenosine uptake. Consistent with the previous literature [6], we also found increased levels of adenosine and cAMP in ticagrelor-treated ACS patients. The underlying mechanisms may involve the inhibitor adenosine uptake mediated by sodium-dependent concentrative nucleoside transporters and sodium-independent ENT [25]. Adenosine is also an important mediator of inhibition of platelet activation, and its effects are mediated via G-protein-coupled adenosine receptors [26, 27]. These receptors modulate the intracellular cAMP levels, an inhibitor of platelet activation [28]. Adenosine and cAMP inhibit the platelet activation through activation of protein kinase A, which phosphorylates specific substrates that are necessary for this process [29]. Our findings demonstrated that ticagrelor had significant off-target properties in ACS patients through a direct action on adenosine and cAMP plasma concentration. Conforming to the previous study [23], ticagrelor achieved a more potent antiplatelet effect compared to clopidogrel as measured by the TEG.

Adenosine and cAMP modulate the inflammatory responses to a variety of stressful conditions and inhibits the release and production of reactive oxygen species and inflammatory mediators [30]. However, the potential anti-inflammatory activity of ticagrelor has not been found in our study. The possible reason was that all the enrolled patients were medicated with statins which had an anti-inflammation effect.

Our results further showed that the APC and cAMP triggered by ticagrelor were positively correlated with ADP-induced platelet aggregation in ACS patients (r=0.68, P<0.001; r=0.30, P=0.002, respectively). A previous investigation involving ACS patients suggested that ticagrelor could augment the coronary blood flow velocity via the incremental APC. The underlying mechanism might be that the increasing adenosine and cAMP levels led to a total decrease in platelet accumulation at the site of vascular injury thus inhibiting the initial rate of platelet accumulation [17, 31, 32]. Adenosine and cAMP serve as a regulator of the rate at which the platelets adhere and detach from a thrombus at a site of vascular injury.

In the PLATO trial, ticagrelor was associated with an increased incidence of dyspnea, which was not accentuated with clopidogrel, 14.5% versus 8.7%, respectively [5]. Our results were consistent with the previous study and demonstrated that the incidence of dyspnea was higher in the ticagrelor group 16.9% compared to the clopidogrel group 5.8%, P=0.028. Gaubert et al. [33] reported that ticagrelor (180 mg) significantly enhanced the sensation of dyspnea, as measured by the Borg scale [34], as a consequence of adenosine infusion in healthy volunteers. Ticagrelor is known to induce dyspnea that is not associated with bronchospasm but through the increasing levels of adenosine [35].

In this observational study, we cannot completely exclude the possible bias by various risk factors and patient characteristics although no statistical difference was observed in the demographic and clinical characteristics of the participants. Firstly, this study was a single-center investigation and sample size calculation was not performed, which might be limiting in order to derive definite conclusions. Secondly, we used the TEG to measure the IPA which might not be sufficient to diagnose fully the response to antiplatelet therapy. Lastly, we only carried out a 30-day follow-up, which might be limiting in drawing a definite conclusion of the clinical benefits of ticagrelor.

In summary, ticagrelor had a potential anti-platelet effect which might be mediated with the increasing adenosine and cAMP plasma concentration according to our study. These findings provide clinical evidence for the adenosine and cAMP on the biological effect in patients with ACS. Further, larger randomized controlled clinical trials will still be necessary to assess whether the ticagrelor induced biological effects can be translated into an improvement on the cardiovascular endpoints in patients with ACS.

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Disclosure of conflict of interest

None.

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References


