Ticagrelor Compared with Clopidogrel Increased Adenosine and Cyclic Adenosine Monophosphate Plasma Concentration in Acute Coronary Syndrome Patients

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Abstract: Ticagrelor produces a more potent antiplatelet effect than clopidogrel and inhibits cellular uptake of adenosine, which is associated with several cardiovascular consequences. We aimed to explore the correlation between adenosine and cyclic adenosine monophosphate (cAMP) plasma concentration and antiplatelet effect by clopidogrel or ticagrelor in patients with acute coronary syndrome (ACS) receiving dual antiplatelet therapy (DAPT). We conducted a prospective, observational and single-centre cohort study enrolling 68 patients with non-ST-segment elevation ACS from January 2016 to May 2016. We monitored the inhibition of platelet aggregation (IPA) and assessed adenosine, adenosine deaminase (ADA) and cAMP plasma concentrations by immunoenzymeassay on admission and 48 hr after coronary angiography. The demographic and clinical data were collected by reviewing their medical records. The two groups exhibited similar baseline characteristics including adenosine, ADA and cAMP. The mean IPA in patients receiving ticagrelor was significantly higher than that in patients receiving clopidogrel (93.5% versus 67.2%; p = 0.000). Also, we observed that patients treated with ticagrelor had a significantly higher increase in levels of adenosine and cAMP compared with those treated with clopidogrel (1.04 (0.86; 1.41) versus 0.04 (−0.25; 0.26); p = 0.029 and 0.78 (−1.67; 1.81) versus 0.60 (−1.91; 4.60); p = 0.037, respectively). And there was a weak correlation between IPA and adenosine as well as cAMP plasma concentration (r = 0.390, p = 0.001 and r = 0.335, p = 0.005, respectively). Ticagrelor increased adenosine and cAMP plasma concentration compared with clopidogrel in patients with ACS.

The P2Y12 receptor antagonists play an important role in the inhibition of platelet aggregation and prevention of atherothrombotic events for the patients with acute coronary syndromes (ACSs) [1]. Ticagrelor, a new kind of P2Y12 receptor antagonist, produced a faster, greater and more consistent antiplatelet effect than clopidogrel [2]. In the PLATO study, ticagrelor reduced the major adverse cardiac and cerebrovascular events (MACCE) compared with the standard treatment with clopidogrel [3]. However, ventricular pauses and dyspnoea were observed in patients treated with ticagrelor. These findings led to the hypothesis that the new P2Y12 receptor antagonist ticagrelor may have a pleiotropic property.

Many studies have concluded that ticagrelor could increase the plasma concentration of adenosine (APC), which might inhibit the cellular uptake of adenosine by the equilibrative nucleoside transporter-1 (ENT-1), leading to an increase in APC [3–5]. As a result of such critical role, studies have suggested that ticagrelor could augment adenosine-induced coronary blood flow velocity and the sensation of dyspnoea [6]. Adenosine is a purine nucleoside produced primarily through the metabolism of adenosine diphosphate (ADP) or adenosine triphosphate by the nucleotidases CD39 and CD73 [7]. Adenosine exerts biological effects on inflammatory regulation of the cardiovascular system and on platelet aggregation through interacting with four types of cellular adenosine receptors (A1, A2A, A2B and A3) [8,9].

All pieces of current evidence of this adenosine-mediated antiplatelet effect by ticagrelor are derived from in vitro studies or from healthy volunteers. Therefore, the role of adenosine metabolism still lacks clinical support [5]. We suggested that the pleiotropic property of ticagrelor could modulate adenosine level, and therefore carried out a prospective, observational and single-centre cohort study in 68 patients with ACS who had never received ticagrelor and clopidogrel therapy previously. The purpose of our study was to investigate whether ticagrelor increased the level of adenosine, adenosine deaminase (ADA) and cyclic adenosine monophosphate (cAMP) compared with clopidogrel in patients with ACS.

Materials and Methods

Study design and population. We performed a prospective, observational and single-centre cohort study enrolling 68 patients with non-ST-segment elevation ACS from January 2016 to May 2016. The protocol was approved by the Ethical Committee of Zhongshan Hospital, Fudan University. The study was conducted in accordance with Good Clinical Practice and in compliance with the Helsinki Declaration. Informed consent was obtained from all the patients before undergoing any study procedure.

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. All the patients were allocated to a ticagrelor group or a clopidogrel group based on the clinician practice or evidence-based medicine. Patients with a history of chest pain lasting for more than 20 min., elevated
cardiac biomarker levels and a change of T-wave on electrocardiogram (ECG) were eligible for inclusion. All patients underwent successful percutaneous coronary intervention (PCI) for reperfusion, and drug-eluting stents were implanted. The exclusion criteria were age less than 18 years, allergies or contraindications to either ticagrelor or clopidogrel, a history of cerebral haemorrhage, increased risk of bleeding and haematologic disorder, severe liver and kidney insufficiency, acute and chronic infection, platelet count <100 \times 10^9 and requirement for coronary artery bypass graft (CABG) surgery after coronary angiography.

The enrolled patients underwent PCI operation through the radial artery route. A loading dose of 180 mg ticagrelor followed by a regimen of 90 mg bidaily was administrated in the ticagrelor group and loading dose of 300 mg clopidogrel followed by a daily regimen of 75 mg in the clopidogrel group. All patients received a bolus of heparin during the PCI procedure.

Individuals who had undergone PCI had an additional detailed characterization including the history of smoking or alcohol consumption, comorbidity disease, haemoglobin, haematocrit, platelet count, alanine aminotransferase (ALT), estimated glomerular filtration rate (eGFR), left ventricular ejection fraction (LVEF) and the number of stents.

Detection of the adenosine, ADA and cAMP plasma concentration. Blood samples were collected by atraumatic venipuncture of the antecubital vein on admission and 48 hr after coronary angiography.

The APC was measured by treatment with adenosine deaminase followed by a multi-step enzymatic approach resulting in the generation of an intermediate that reacts with the adenosine probe to form a fluorescent product. The fluorescent product (Biovision, Milpitas, CA, USA) was measured at Ex/Em = 355/587 nm with a detection range of 2–80 pmol. ADA was measured by the formation of ammonium (Jiancheng, Nanjing, China) with absorbance at 560 nm after incubation. The cAMP assay (R&D Systems, Minneapolis, MN, USA) was based on the competitive binding technique. A monoclonal antibody specific for cAMP interacted with the goat antimouse antibody coated onto the microplate. After a wash to remove excess monoclonal antibody, cAMP in the sample competed with a fixed amount of horseradish peroxidase-labeled cAMP for sites on the monoclonal antibody. This was followed by another wash to remove the excess conjugate and the unbound sample. A substrate solution was added to the wells to determine the bound enzyme activity. The colour development was quenched, and the absorbance was measured at 450 nm. The intensity of the colour was inversely proportional to the concentration of cAMP in the sample. The detectable dose of cAMP ranged from 0.42 pmol/mL to 8.57 pmol/mL.

Clinical end-point. Major adverse cardiovascular events (MACE), 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 in a 30-day follow-up. Ticagrelor-induced adverse drug reactions of dyspnoea and atrial ventricular block for each patient according to the PLATO clinical trial [3] were monitored.

Sample size calculation. Based on pilot data analysis, we suggested that ticagrelor could result in the increase in adenosine plasma concentration compared with clopidogrel in the patients with ACS. A power of 85% and a two-sided level of 0.05 and ≥62 patients in total were required to reach statistical significance based on the above assumptions. We assumed a loss rate of 10%. Therefore, the total number of patients enrolled was estimated to be 68.

Statistical analysis. Data are presented as mean ± S.D. for numeric and categorical variables. Comparisons of continuous variables between the two groups were performed by Student’s t-test and comparisons of categorical variables by chi-square or Fisher’s exact test (expressed as an odds ratio). Data were analysed using IBM SPSS Statistics 19.0 software. A p Value <0.05 was considered statistically significant.

Results

Baseline characteristics of the studied population and levels of adenosine, ADA and cAMP.

This study included 68 patients. There were no differences in demographic and clinical baseline characteristics between the two patient groups (table 1).

The levels of adenosine, ADA and cAMP were analysed by multi-step enzymatic approach on admission. There were no significant differences in baseline levels between the two groups (table 2).

Variation of adenosine, ADA and cAMP levels after 48-hr coronary angiography.

To evaluate the influence of different ADP-induced antiplatelet effects on the levels of adenosine, ADA and cAMP, we calculated the change in levels of these between baseline and after coronary angiography at 48 hr (table 2).

Regarding the changes in adenosine and cAMP levels, we observed a significant difference between the patients treated with ticagrelor and clopidogrel (p = 0.029 and p = 0.037, respectively) (table 2). We noticed a significantly higher increase in the levels of adenosine and cAMP in the ticagrelor group. However, there was no significant difference observed with the changes in ADA levels between the two groups (p = 0.749) (table 2).

Considering the potential effects of adenosine and cAMP might influence the antiplatelet treatment, we assessed the correlation between the laboratory parameters and IPA after coronary angiography at 48 hr. Our results suggested that there was a weak correlation between the IPA and adenosine as well as cAMP plasma concentration (r = 0.390, p = 0.001; r = 0.335, p = 0.005, respectively; figs 1 and 2).

Clinical end-points.

The rate of MACE at 1 month after PCI operation was 2.9% in the ticagrelor group and 5.8% in the clopidogrel group. There was no significant difference in rehospitalization ratio of the two groups (p = 0.55). In addition, no patients had significant (BARC > 2) bleeding, dyspnoea and atrial ventricular block during the follow-up.

Discussion

In the PLATO trial, ticagrelor reduced the rate of mortality from vascular causes as compared with clopidogrel [3].
Regarding antiplatelet effect, ticagrelor is more potent than clopidogrel and produces a rapid and robust inhibition of platelet aggregation [12]. Many studies have shown that ticagrelor could inhibit the ENT-1 channel to increase APC, which then inhibits platelet function via the A2A receptor, as well as inhibiting the platelet P2Y12 receptor for ADP in healthy volunteers and animals [4,13]. In agreement with the previous evidence, our study demonstrated that ticagrelor was associated with an increased adenosine and cAMP plasma concentration in patients with ACS. This effect on adenosine and cAMP may participate in the so-called pleiotropic property of ticagrelor.

In our study, we found that the ADP-induced IPA in the ticagrelor-treated group was higher than in the clopidogrel group. In the ONSET/OFFSET clinical trial [2], ticagrelor achieved higher IPA compared with clopidogrel after loading dose. Twenty-four hours after the last dose, the mean IPA was 58% for ticagrelor versus 52% for clopidogrel. Moreover, in the RESPOND clinical trial [14], ticagrelor improved the

### Table 1.

| Characteristics | Ticagrelor (n = 34) | Clopidogrel (n = 34) | p Value |
|-----------------|---------------------|----------------------|---------|
| Age, years (mean ± S.D.) | 63.2 ± 9.1 | 65.8 ± 9.4 | 0.265 |
| Males (%) | 94.1% | 85.3% | 0.427 |
| BMI (kg/m²) (mean ± S.D.) | 24.3 ± 1.1 | 23.3 ± 2.9 | 0.379 |
| Cardiovascular risk factor | | | |
| Smoke (%) | 58.8 | 55.9 | 1.000 |
| Hypercholesterolaemia (%) | 11.7 | 14.7 | 0.720 |
| Hypertension (%) | 61.7 | 55.8 | 0.806 |
| Diabetes mellitus (%) | 23.5 | 20.6 | 0.770 |
| Coronary artery disease extent | | | |
| 1 vessel disease (%) | 52.9 | 50.0 | |
| 2 vessel disease (%) | 23.5 | 32.3 | |
| 3 vessel disease (%) | 23.6 | 17.7 | |
| Number of stents (mean ± S.D.) | 1.68 ± 1.43 | 1.59 ± 1.13 | 0.779 |
| Medication on admission | | | |
| Aspirin (%) | 94.1 | 100.0 | 0.493 |
| GP IIb/IIIa receptor antagonist (%) | 73.5 | 70.5 | 0.786 |
| Statin (%) | 100.0 | 100.0 | 1.000 |
| ACEI/ARB (%) | 97.0 | 94.1 | 0.555 |
| β-Blocker (%) | 91.7 | 94.1 | 0.642 |
| PPI (%) | 61.7 | 61.7 | 1.000 |
| Biological parameters (mean ± S.D.) | | | |
| Haemoglobin | 129.9 ± 17.1 | 136.0 ± 20.2 | 0.260 |
| Haematocrit, % | 38.8 ± 4.8 | 40.3 ± 5.1 | 0.219 |
| Platelet | 266.5 ± 80.9 | 238.9 ± 47.3 | 0.091 |
| Creatinine clearance, mL/min. | 87.2 ± 16.2 | 80.8 ± 20.4 | 0.151 |
| Uric acid | 359.5 ± 104.7 | 345.0 ± 122.6 | 0.602 |
| Inhibitor of Platelet Aggregation, % | 93.5 ± 5.6 | 67.2 ± 22.6 | 0.000* |

BMI, body mass index; GP, glucose protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; PPI, proton pump inhibitor.

*p<0.05.

### Table 2.

| Variables | Time | Ticagrelor | Clopidogrel | p Value |
|-----------|------|------------|-------------|---------|
| Adenosine | Baseline | 2.66 ± 0.58 | 2.63 ± 0.54 | 0.793 |
|            | Changes | 1.04 (0.86; 1.41) | 0.04 (–0.25; 0.26) | 0.029* |
| ADA       | Baseline | 10.85 ± 3.99 | 11.67 ± 3.36 | 0.361 |
|            | Changes | 0.04 (–0.25; 0.26) | 0.78 (–1.66; 1.82) | 0.749 |
| cAMP      | Baseline | 19.12 ± 7.51 | 17.57 ± 9.40 | 0.454 |
|            | Changes | 0.78 (–1.67; 1.81) | 0.60 (–1.91; 4.60) | 0.037* |

*p<0.05.

**Fig. 1.** The correlation between APC and IPA.
antiplatelet effect for non-responsiveness to clopidogrel. In our study, the mean IPA of ticagrelor was 93.5% higher than the mean IPA of clopidogrel (67.2%). Ticagrelor does not require metabolic activation for antiplatelet activity and binds reversibly to the P2Y12 receptor [15]. After administration of GP IIb/IIIa receptor antagonist, the IPA of the ticagrelor group was higher than that reported in previous studies. This may be because GP IIb/IIIa receptors bind to fibrinogen, which serves as the ‘final common pathway’ in platelet aggregation, leading to higher IPA levels [16].

Adenosine is a purine nucleoside produced primarily through the metabolism of ADP or adenosine triphosphate by the nucleotidases CD39 and CD73 [7]. Many studies have previously provided the evidence that ticagrelor could inhibit the uptake of adenosine via inhibiting ENT [17]. Adenosine exerts its biological effects by interacting with G-protein-coupled receptors, which stimulates adenylyl cyclase and thus increases cAMP plasma concentration [18]. Consistent with the previous literature, we also discovered the increased levels of adenosine and cAMP in ticagrelor-treated patients [9]. Adenosine and cAMP inhibit the platelet activation through activation of protein kinase A, which phosphorylates specific substrates that are necessary for this process [19]. Our study showed that ticagrelor did not influence the ADA plasma concentration compared with clopidogrel, which is generally consistent with the finding that the serum of patients treated with ticagrelor had no direct effect on adenosine receptor A1 or A2A and did not affect ADA [9].

Our results further showed that adenosine and cAMP triggered by ticagrelor were weakly correlated with ADP-induced platelet aggregation in patients with ACS, suggesting that the adenosine and cAMP were independent of antiplatelet effect. The main mechanism of the pathophysiology of ACS was platelet aggregation [20]. Ticagrelor had a potential inhibition on ADP-induced platelet aggregation by antagonizing P2Y12 receptor. In addition, many present studies demonstrated that ticagrelor had a pleiotropic property that might be mediated by adenosine. Ticagrelor could increase the adenosine and cAMP plasma concentration by inhibiting the uptake through the ENT. Adenosine and cAMP inhibit platelet activation mainly via A2A receptor but also via A2B receptor [21,22]. The main mechanism of the weak correlation with adenosine and antiplatelet effect might be that ticagrelor had a low affinity on platelet A2A receptors [19]. Bonello et al. [19] previously provided the evidence that the adenosine plasma concentration had no correlation with the platelet reactivity inhibition.

In the PLATO trial, ticagrelor was associated with an increased incidence of dyspnoea, which was not accentuated with clopidogrel, 14.5% versus 8.7%, respectively [3]. Ticagrelor is known to induce dyspnoea that is not associated with bronchospasm but through the increasing levels of adenosine [23]. Our research demonstrated that no patients had dyspnoea during the follow-up. Due to the relatively small sample size, further research is needed to validate the findings of this study.

In this observational study, we could not completely exclude the possible bias introduced by various risk factors and patient characteristics, even though no statistical difference was observed in the demographic and clinical characteristics of the study participants. Firstly, this study was a single-centre investigation and the sample size was small, which might be limited for deriving definite conclusions. Secondly, we used the TEG to measure the IPA, which might not be sufficient to fully diagnose the response to antiplatelet therapy. Lastly, we only carried out a 30-day follow-up, which might be limited in drawing a definite conclusion of the clinical benefits of ticagrelor.

In summary, we found that ticagrelor could increase the adenosine and cAMP plasma concentration in our study. These findings provide clinical evidence for adenosine and cAMP on the biological effect in patients with ACS. Further, larger randomized, controlled clinical trials will be necessary to assess whether the ticagrelor-induced biological effects can be translated into an improvement of the cardiovascular endpoints in patients with ACS.

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Compliance with Ethical Standards
The study protocol was approved by the Ethics Committee of Zhongshan Hospital, Fudan University. It was conducted in accordance with Good Clinical Practice and in compliance with the Helsinki Declaration. Informed consent was obtained from all patients before undergoing any study procedure.

Author Contributions
Xiaoye Li and Qianzhou Lv designed the study, performed the research, analysed the data and wrote the manuscript. Ying Xue, Qibing Wang and Jiahui Chen performed the research and wrote the manuscript.

Conflict of Interest
The authors declare that they have no conflict of interest.
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