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Ticagrelor Increases Adenosine Plasma Concentration in Patients With an Acute Coronary Syndrome

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Objectives	This study aimed to investigate the impact of ticagrelor on adenosine plasma concentration (APC) in acute coronary syndrome (ACS) patients.
Background	Ticagrelor is a direct-acting P2Y ₁₂ -adenosine diphosphate receptor blocker. The clinical benefit of ticagrelor compared with clopidogrel in ACS patients suggests that the drug has non-platelet-directed properties. Animal and in vitro models suggested that the “pleiotropic” properties of ticagrelor may be related to an interaction with adenosine metabolism.
Methods	We prospectively randomized 60 ACS patients to receive ticagrelor or clopidogrel. The APC was measured by liquid chromatography. To assess the mechanism of APC variation, we measured adenosine deaminase concentration, adenosine uptake by red blood cells, and cyclic adenosine monophosphate production by cells overexpressing adenosine receptors. The P2Y ₁₂ -adenosine diphosphate receptor blockade was assessed by the vasodilator-stimulated phosphoprotein index.
Results	Patients receiving ticagrelor had significantly higher APC than patients receiving clopidogrel (1.5 mM [interquartile range: 0.98 to 1.7 mM] vs. 0.68 mM [interquartile range: 0.49 to 0.78 mM]; $p < 0.01$). The APC was not correlated with vasodilator-stimulated phosphoprotein ($p \geq 0.16$). Serum-containing ticagrelor inhibited adenosine uptake by red blood cells compared with clopidogrel or controls ($p < 0.01$ for both comparisons). Adenosine deaminase activity was similar in serum of patients receiving clopidogrel or ticagrelor ($p \geq 0.1$). Ticagrelor and clopidogrel had no direct impact on adenosine receptors ($p \geq$ not significant).
Conclusions	Ticagrelor increases APC in ACS patients compared with clopidogrel by inhibiting adenosine uptake by red blood cells. (

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Ticagrelor is a direct-acting and reversible P2Y₁₂-adenosine diphosphate (ADP) receptor blocker that has demonstrated higher biological efficacy than clopidogrel (1). Its higher potency led to improved prognosis in acute coronary

syndrome (ACS) patients (2). Interestingly, unlike in previous trials of P2Y₁₂-ADP receptor blockers, in the PLATO (Platelet Inhibition and Patient Outcomes) trial the benefit of ticagrelor has continued to grow, and cardiovascular mortality has been significantly reduced (2–4). These observations led to the hypothesis that ticagrelor has pleiotropic properties and suggested some novel nonplatelet-directed mechanisms of action.

Adenosine is released in the plasma by endothelial cells and myocytes during ischemia, hypoxia, or oxidative stress (5,6). Most of the plasma adenosine is quickly taken up by red blood cells (RBC) through a facilitated diffusion transport system (7) or converted into inosine by adenosine deaminase activity (ADA) (8). An increase in APC may, therefore, result from the inhibition of RBC uptake, reduced ADA, or both. Increased APC impacts the cardiovascular system by purinergic receptors, named A_1 , A_{2A} , A_{2B} , or A_3 depending on their pharmacological properties (9).

In experimental studies, ticagrelor has been shown to increase adenosine-induced physiological response by shifting the dose-response curve for adenosine-induced coronary blood flow velocity to the left (10). In addition, in vitro experiments suggested that ticagrelor inhibited adenosine re-uptake by human RBC (11). However, these observations were made in a dog model and in vitro. Therefore, we aimed to investigate in ACS patients the effects of ticagrelor on APC.

Methods

We performed a single-center, prospective, open-label, randomized trial enrolling patients with intermediate- or high-risk non-ST-segment elevation ACS. Twenty healthy subjects without medication (12 men and 8 women) from our medical staff served as control group.

The present study was approved by the institutional review board and agrees with the declaration of Helsinki. All patients gave informed consent. They were randomly allocated to ticagrelor or clopidogrel according to a sequence generated using R software (blockrand package, R Foundation for Statistical Computing, Vienna, Austria).

Blood samples were collected before coronary angiography and 6 h after a P2Y₁₂-ADP receptor antagonist loading dose.

For antiplatelet therapy, a loading dose of 180 mg ticagrelor followed by 90 mg twice-daily was used in the ticagrelor group, and a loading dose of 600 mg clopidogrel followed by 75 mg daily was used in the clopidogrel group. In addition, all patients received a loading dose of 250 mg aspirin followed by 75 mg daily.

Exclusion criteria were New York Heart Association functional class greater than or equal to III, cardiac arrest, contraindications to antiplatelet therapy, treatment with a P2Y₁₂-ADP receptor antagonist for <1 month, a platelet count <100 g/l, history of bleeding diathesis, history of hemorrhagic stroke, stroke in the preceding year, recent surgery (preceding month), use of medication having known interference with ticagrelor, and bradycardia.

Vasodilator-stimulated phosphoprotein measurement. The vasodilator-stimulated phosphoprotein (VASP) index phosphorylation analysis was performed within 24 h of blood collection using platelet VASP kits (Diagnostica Stago, Asnières, France) (12). The VASP index was calculated from the mean fluorescence intensity (MFI), assessed by flow cytometry, of samples incubated with either prostaglandin

(PG) E1 or PGE1 and ADP according to the following formula:

$$\text{VASP} \frac{1}{4} \left[\frac{\text{MFI(PGE1)} + \text{MFI(PGE1)} + \text{MFI(PGE1)} + \text{MFI(PGE1)}}{\text{MFI(PGE1)}} \right]$$

100. Patients with a VASP index

50% were considered to have high on-treatment platelet reactivity (PR) (13). Adenosine plasma concentration measurement. Blood samples (3 ml) were collected and processed as described (14,15). Fresh whole blood was collected in tubes containing a stop solution

to prevent RBC uptake and degradation of adenosine by ADA. The stop solution is required to prevent adenosine degradation, which is very quick otherwise. The stop solution was composed of dipyridamole, 0.2 mM; ethylenediaminetetraacetic acid disodium, 4 mM; erythro-9-(2-hydroxy-3-nonyl adenine), 5 mM; alpha,beta-methyleneadenosine 5^β-diphosphate, 79 mM; 2^β-deoxycoformycin, 10 mg/ml; and heparin sulfate, 1 IU/ml. After centrifugation, supernatants were deproteinized, and APC was measured by high-performance liquid chromatography, as previously described (14,15).

Adenosine deaminase activity. Fresh serum was pipetted off after centrifugation of whole blood. The ADA was evaluated by the ammonium formed (Beckman DX, Beckman Coulter, Brea, California) after incubation (40 min at 37°C) of 125 μl serum with 750 μl adenosine (28 mM), as previously described (16).

Adenosine uptake by RBC. Samples of fresh whole blood (1 ml) of patients and controls were collected on ethylenediaminetetraacetic acid tubes without stop solution. Then, 1 nmol of adenosine was added. Samples were continuously mixed with a vortex mixer for 60 s. Uptake by RBC was stopped by adding 500 μl cold stop solution at time 0 (T0) and after 15 s (T15), 30 s (T30), and 60 s (T60). Samples were immediately centrifuged (4°C, 1,500g for 10 min), and APC was measured (15–17).

Adenosine receptor activity. To evaluate a potential adenosine receptor's agonist property of ticagrelor or clopidogrel derivatives, we assessed the impact of the serum of patients on cyclic adenosine monophosphate (cAMP) production of recombinant cell lines overexpressing the A_1 or A_{2A} receptor (CHO Chem 3 cells, Millipore, Billerica, Massachusetts).

Cyclic AMP concentration determination. Fresh serum of patients and controls were pipetted off after centrifugation of whole blood. No stop solution was used as adenosine is quickly degraded by ADA, thus allowing assessment of the ability of drug derivatives to act on receptors. Modulation of cAMP production by the patient's serum was tested and compared with serum controls and with serum

Abbreviations and Acronyms

ACS	= acute coronary syndrome(s)
ADA	= adenosine deaminase activity
ADP	= adenosine diphosphate
APC	= adenosine plasma concentration
cAMP	= cyclic adenosine monophosphate
MFI	= mean fluorescence intensity
PG	= prostaglandin
PR	= platelet reactivity
RBC	= red blood cells
VASP	= vasodilator-stimulated phosphoprotein

Table 1 Baseline Characteristics of Study Population								
	Ticagrelor (n ¼ 30)		Clopidogrel (n ¼ 30)		p Value			
Age, yrs	62	12	23	64	12	22	0.5	
Male	(76.7)		27.8	(73.3)		27.6	0.9	
Body mass index, kg/m ²	5.1			4.6			0.8	
Cardiovascular risk factors								
Smoking	9 (30)			7 (23.3)			0.8	
Hypercholesterolemia	17 (56.7)			21 (70)			0.5	
Diabetes mellitus	11 (36.7)			12 (40)			0.9	
Hypertension	15 (50)			16 (53.3)			0.9	
Family history of CAD	7 (23.3)			5 (16.7)			0.6	
Clinical setting								
NSTEMI	19 (63.3)			19 (63.3)			1	
Unstable angina	11 (36.7)			11 (36.7)				
Medication on admission								
Proton pump inhibitor	10 (34.5)			10 (34.5)			1	
Aspirin	7 (24.1)			14 (46.7)			0.1	
Beta-blocker	8 (27)			10 (34.5)			0.6	
Statin	12 (40)			12 (40)			1	
Angiography and intervention								
Number of diseased vessels								
1	14 (46.7)			8 (26.7)			0.8	
2	7 (23.3)			13 (43.3)				
3	9 (30)			9 (30)				
Biology								
Leukocytes, g/l	9.4		3.3	8.6		2.8	0.4	
Hemoglobin, g/dl	14		1.8	13.8		1.9	0.7	
Platelets, 10 ⁹ /l	247		64	243		44	0.7	
Fibrinogen, g/l	3.6		1	3.6		0.8	0.9	
Creatinine, mmol/l	24		12.3	89		27	17	0.8
CRP, mg/l	18			20			0.4	
VASP index, %	16.5		15	46.9		22.3	<0.0001	

Values are mean SD or n (%).

CAD ¼ coronary artery disease; CRP ¼ C-reactive protein; NSTEMI ¼ non-ST-segment elevation myocardial infarction; VASP ¼ vasodilator-stimulated phosphoprotein.

containing cyclopentyladenosine (CPA), an A₁ receptor agonist, or CGS21680, an A_{2A} receptor agonist, in a nanomolar concentration. The cAMP concentration was determined by immunoassay (cAMP Biotrak EIA, Amersham, Orsay, France), as previously described (18).

C-reactive protein. To evaluate a potential link between inflammation and adenosine metabolism, we measured C-reactive protein (CRP) using Beckman DX apparatus.

Clinical endpoints. Major adverse cardiovascular events, including cardiovascular death, myocardial infarction, urgent revascularization, and stroke at 1 month, were recorded. Bleeding was measured using the TIMI (Thrombolysis In Myocardial Infarction) study classification. Dyspnea was also recorded.

Statistical analysis. Analyses were performed with R free software, version 2.8.1 (R Foundation for Statistical Computing). Continuous variables are expressed as mean SD or median and interquartile range (IQR). They were compared using the nonparametric Mann-Whitney U test. The nonparametric Wilcoxon test was used for intraindividual comparisons. Multiple comparisons were made among the

4 groups using the multiple comparisons Mann-Whitney U test with Bonferroni corrections. Spearman's r coefficient of correlation was used for correlation studies.

Results

The baseline characteristics of the study population are given in Table 1.

APC after P2Y₁₂-ADP receptor loading dose. The APC was more than 2-fold higher in patients taking ticagrelor than in patients taking clopidogrel (1.5 mM [IQR: 0.98 to 1.7 mM] vs. 0.68 mM [IQR: 0.49 to 0.78 mM]; p < 0.01) or controls (0.6 mM [IQR: 0.5 to 0.8 mM]; p < 0.01) (Fig. 1). No statistical difference was observed between patients under clopidogrel and controls.

Mechanism of APC increase. EFFECTS OF PATIENTS' SERUM ON ADA. The patients in the 2 treatment groups did not differ significantly regarding ADA (p ¼ 0.1). The ADA was higher in serum of patients in the ticagrelor group and clopidogrel group (15 IU [IQR: 12.6 to 18.2 IU] and 13.5 IU [IQR: 12 to 16 IU], respectively) than in control subjects

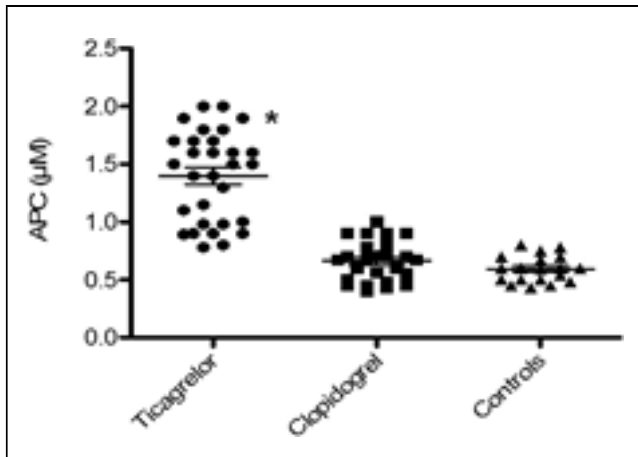


Figure 1 Comparisons of APC in Ticagrelor, Clopidogrel, and Control Groups

*Indicates $p < 0.01$ for ticagrelor group versus control group or clopidogrel group. APC = adenosine plasma concentration.

(8 IU [IQR: 7.1 to 9 IU]; $p < 0.01$ for both groups) (Fig. 2). The ADA weakly correlated with CRP ($r = 0.47$; $p < 0.01$) (Fig. 3).

EFFECTS OF PATIENTS' SERUM ON cAMP PRODUCTION. Regarding the production of cAMP by cells expressing the adenosine receptors, no difference was observed between serum of patients in the ticagrelor group and in the clopidogrel group compared with controls ($p = 0.7$ and $p = 0.1$ for A₁ receptors, respectively; and $p = 0.6$ and $p = 0.1$ for A_{2A} receptors, respectively) (Fig. 4).

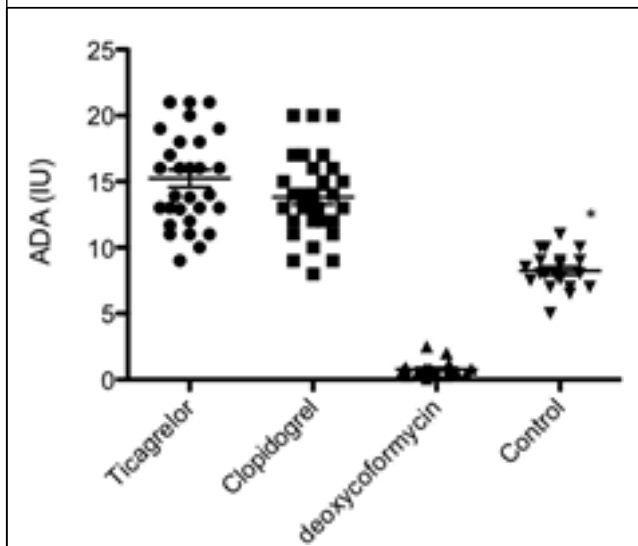


Figure 2 ADA of Serum-Containing Drugs

Deoxycoformycin was used as adenosine deaminase activity (ADA) inhibition control value. *Indicates $p < 0.01$ control group versus ticagrelor or clopidogrel group.

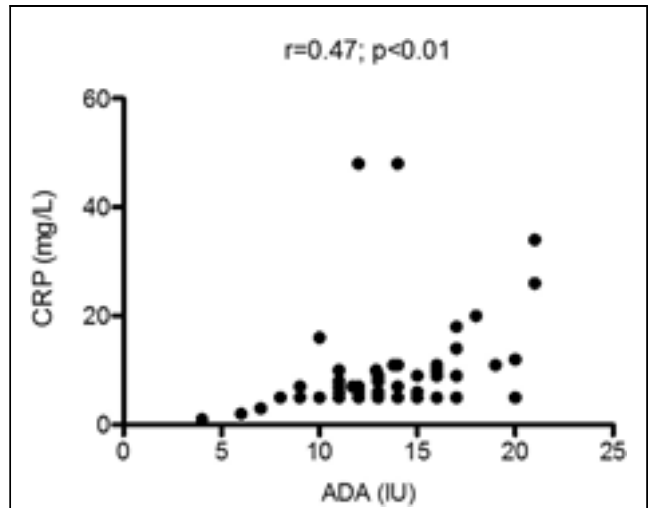


Figure 3 Correlation Between ADA and CRP

Correlation (Spearman's r) between adenosine deaminase activity (ADA) and C-reactive protein (CRP).

EFFECTS OF PATIENTS' SERUM ON ADENOSINE UPTAKE BY RBC. Ticagrelor significantly inhibited adenosine uptake by RBC compared with clopidogrel ($p < 0.001$ at all times) and with controls ($p < 0.001$ at all times). Clopidogrel did not impact adenosine uptake by RBC compared with controls ($p =$ not significant at all times) (Fig. 5).

PR and relationship with APC. Ticagrelor induced a significantly higher PR inhibition than clopidogrel, as measured by the VASP index, after P2Y₁₂-ADP receptor loading dose (14.7% [IQR: 9.2% to 19.8%] vs. 44% [IQR: 33.5% to 64.2%]; $p < 0.001$). The rate of patients with high on-treatment PR after ticagrelor loading dose was significantly lower compared with clopidogrel loading dose (3% vs. 40%; $p < 0.001$). Finally, PR inhibition and adenosine plasma concentration were not correlated ($r = 0.27$; $p = 0.2$).

Clinical endpoints. No ischemic or bleeding events were recorded within 1 month. Dyspnea was present in 7 patients in the ticagrelor group and in 5 patients in the clopidogrel group ($p = 0.6$).

Discussion

The present study is the first to demonstrate a significant increase in APC in ACS patients treated with ticagrelor compared with clopidogrel. In addition, we observed that this property of ticagrelor was related to the inhibition of adenosine uptake by RBC. These findings demonstrate that ticagrelor has significant off-target properties in ACS patients through a direct action on APC. Finally, in line with previous studies, ticagrelor achieved a more potent PR inhibition compared with clopidogrel as measured by the VASP index (1).

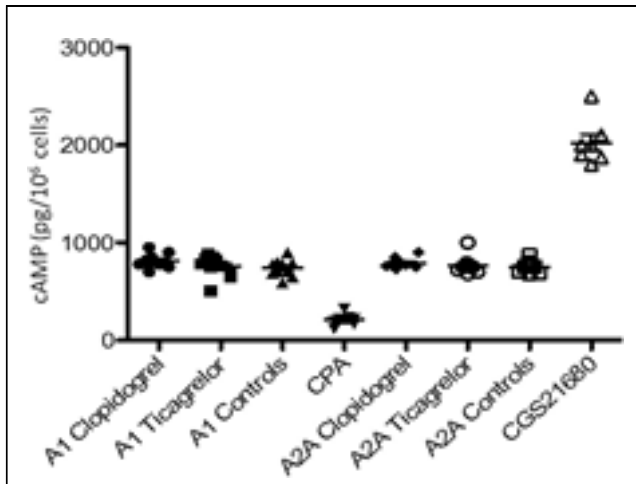


Figure 4 Production of cAMP by Chem Cells Overexpressing A₁ or A_{2A} Receptors

Production of cyclic adenosine monophosphate (cAMP) production in the presence of serum of patients treated with clopidogrel or ticagrelor or with serum control subjects was similar for the A₁ and A_{2A} receptors. Cyclopentyladenosine (CPA) inhibited, whereas CGS21680 increased, cAMP production.

A previous investigation involving healthy volunteers suggested that ticagrelor increases an adenosine-induced physiological response by shifting the dose-response curve for adenosine-induced coronary blood-flow velocity to the left (10). Accordingly, in an animal experiment, van Giezen et al. (11) observed that ticagrelor enhances adenosine-mediated responses on coronary blood flow. Furthermore,

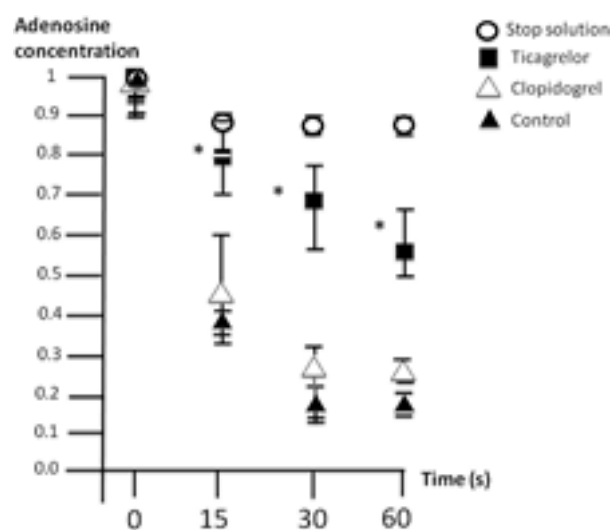


Figure 5 Effects of Drugs on Adenosine Uptake by RBC

At all times, serum of patients receiving ticagrelor (solid squares) was associated with a higher adenosine plasma concentration compared with the serum of clopidogrel patients (open triangles) or control subjects (solid triangles). Open circles ¼ stop solution. *p < 0.001 ticagrelor group versus clopidogrel group. RBC ¼ red blood cells.

those researchers suggested that this effect may be related to the inhibition of adenosine uptake by RBC. Consistent with these preliminary findings, the present study demonstrated that ticagrelor increased APC in ACS patients.

Our study has several additional originalities. First, it was performed in ACS patients, and thus it accurately reflects the effect of ticagrelor in patients with ischemia and coronary artery disease. Second, we compared ticagrelor with clopidogrel, unlike previous studies that used placebo as control value (10). Finally, we also focused on the mechanism of APC increase. In the present study, this property of ticagrelor over APC was independent of P2Y₁₂-ADP receptor blockade. The serum of patients treated with ticagrelor had no direct effect on adenosine receptor A₁ or A_{2A} and did not affect ADA. Finally, we demonstrated that the serum of ACS patients treated with ticagrelor inhibited adenosine uptake by RBC, leading to the increase in APC similar to what was observed for dipyridamole (19,20).

The present results suggest that the “pleiotropic” properties of ticagrelor could in part be mediated by the increased APC. Adenosine has multiple properties involving blood, vessels, and ischemic damage. First, it inhibits platelet aggregation through the activation of A_{2A} receptors. Second, several lines of evidence confirmed the vasodilatory properties of adenosine on arterial beds (21). These properties could be related to the activation of low-affinity A_{2A} receptors through the increase in APC, as these receptors are strongly implicated in coronary blood flow regulation (21,22). Accordingly, the APC level measured in the present study in the ticagrelor group is compatible with the activation of the adenosine A_{2A} low affinity receptors (21). These properties of adenosine may be particularly important in the context of diseased vessels and hypoxia associated with ACS. However, the role of adenosine in protection of the ischemic heart remains controversial, and the long-term impact of high APC on coronary artery disease remains to be determined (23).

Ticagrelor was shown to have specific side effects, bradycardia and dyspnea, which could also be triggered by APC increase (2). However, the present study was not powered to explore a link between APC and these side effects.

Conclusions

Ticagrelor is associated with a significant increase in APC in ACS patients that is related to the inhibition of adenosine re-uptake by RBC. This property of ticagrelor on APC may be responsible for the so-called “pleiotropic” properties of the drug and may contribute to its side effects.

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