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Ticagrelor Enhances Adenosine-Induced Coronary Vasodilatory Responses in Humans

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Objectives This study was undertaken to determine if ticagrelor augments adenosine-induced coronary blood flow and the sensation of dyspnea in human subjects.

Background Ticagrelor is a $P2Y_{12}$ receptor antagonist that showed superior clinical benefit versus clopidogrel in a phase III trial (PLATO [Platelet Inhibition and Patient Outcomes]). Ticagrelor has been shown to inhibit cell uptake of adenosine and enhance adenosine-mediated hyperemia responses in a dog model.

Methods In this double-blind, placebo-controlled study, 40 healthy male subjects were randomized to receive a single dose of ticagrelor (180 mg) or placebo in a crossover fashion. Coronary blood flow velocity (CBFV) was measured by using transthoracic Doppler echocardiography at rest after multiple stepwise adenosine infusions given before and after study drug, and again after the infusion of theophylline.

Results Ticagrelor significantly increased the area under the curve of CBFV versus the adenosine dose compared with placebo ($p = 0.008$). There was a significant correlation between ticagrelor plasma concentrations and increases in the area under the curve ($p < 0.001$). In both treatment groups, the adenosine-induced increase in CBFV was significantly attenuated by theophylline, with no significant differences between subjects receiving ticagrelor or placebo ($p = 0.39$). Furthermore, ticagrelor significantly enhanced the sensation of dyspnea during adenosine infusion, and the effects were diminished by theophylline.

Conclusions Ticagrelor enhanced adenosine-induced CBFV and the sensation of dyspnea in these healthy male subjects via an adenosine-mediated mechanism. (Study to Assess the Effect of Ticagrelor on Coronary Blood Flow in Healthy Male Subjects; NCT01226602) (J Am Coll Cardiol 2013;61:723–7) © 2013 by the American College of Cardiology Foundation

Ticagrelor, a novel, oral, direct-acting, reversibly binding $P2Y_{12}$ receptor antagonist, is approved for the treatment of patients with acute coronary syndrome (1). In the PLATO (Platelet Inhibition and Patient Outcomes) trial, ticagrelor significantly reduced the incidence of myocardial infarction, stroke, or death from vascular causes, compared with standard treatment with clopidogrel (2). In the same study, dyspnea and asymptomatic ventricular pauses were more common in patients receiving ticagrelor than in those receiving clopidogrel.

It has been shown that ticagrelor can inhibit adenosine cell uptake, likely through inhibition of the equilibrative nucleoside transporter 1 (3). Ticagrelor also significantly and dose dependently augmented adenosine-mediated coronary blood flow increases in a dog model (3). These findings could suggest increased local adenosine levels in patients treated with ticagrelor because both dyspnea and ventricular pauses are known effects of adenosine (4,5).

The goal of the current study was to determine if ticagrelor, at a clinically relevant dose, can augment adenosine-induced physiological responses, coronary blood flow velocity (CBFV), and dyspnea in healthy human subjects.

Methods

The study was approved by the Regional Ethical Review Board in Gothenburg, Sweden, and was conducted in accordance with the Declaration of Helsinki. Data management and monitoring were performed by Quintiles AB, Uppsala, Sweden.

Study population. Forty healthy male subjects age 18 to 40 years with a body mass index of 18 to 30 kg/m^2 and weighing 50 to 100 kg provided written informed consent before participating in the study.

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Abbreviations and Acronyms

AUC = area under the curve
CBFV = coronary blood flow velocity
CI = confidence interval
CV% = coefficient of variation
LAD = left anterior descending coronary artery

Study design. This was a double-blind, placebo-controlled, crossover study. After screening, subjects were randomized to receive ticagrelor (180 mg) or placebo. After a washout period of 6 to 21 days, subjects received the alternative regimen (Fig. 1A). They underwent an overnight fast and were required to abstain from caffeine for 24 h before study drug administration.

Subjects received multiple intravenous adenosine infusions with the use of a stepwise dosing protocol: 0, 50, 80, 110, and 140 μg/kg/min (Fig. 1B). Adenosine infusions were given predose, repeated 2 h postdose, and again 10 min after a 20-min intravenous infusion of theophylline (5 mg/kg).

Assessments. CBFV. CBFV was measured in the left anterior descending coronary artery (LAD) before and during the different adenosine infusions (ITEM Development AB, Stocksund, Sweden) by using a Siemens Acuson platform equipped with a 4V1C transducer with 3.5 MHz color and 1.75 MHz spectral Doppler frequency (Acuson Sequoia 512, Siemens, Mountain View, California) (6). To ensure CBFV was measured in the same LAD segment, trough repeated measurements, the surface anatomic position, degree of rotation of the transducer, and the LAD position relative to the left ventricle were carefully documented at the first visit. Cine loops and Doppler images were stored for offline measurements by using Image Arena 2.9.1 (TomTec Imaging Systems GmbH, Unterschleissheim, Germany). Mean diastolic flow velocity was calculated by manually tracing the diastolic flow velocity signal. Baseline CBFV values were calculated by using the mean value of 3 representative heart beats. The mean hyperemic CBFV for each adenosine dose was calculated as the mean of the 3 highest CBFV values.

TICAGRELOR EXPOSURE. Plasma concentrations of ticagrelor and its main circulating metabolite, AR-C124910XX, were measured in venous blood collected 2 h postdose. After protein precipitation, plasma concentrations of ticagrelor and AR-C124910XX were analyzed by using liquid chromatography mass spectrometry. The lower limits of quantification for ticagrelor and AR-C124910XX were 5 and 2.5 ng/ml, respectively (7).

DYSPNEA. Subjects were trained to self-report the sensation of dyspnea after each adenosine dose by using the Borg scale; the scale is scored from 1 (no sensation of dyspnea) to 10 (maximum sensation of dyspnea).

Statistical analysis. All data were summarized by using SAS version 8.02 and R version 2.13.0. The effect of ticagrelor compared with placebo on the area under the curve (AUC) of CBFV versus the adenosine dose (primary endpoint) was estimated by using a mixed-model analysis of variance. The model included the log AUC change with ticagrelor and placebo as the response variable; the log pre-dose

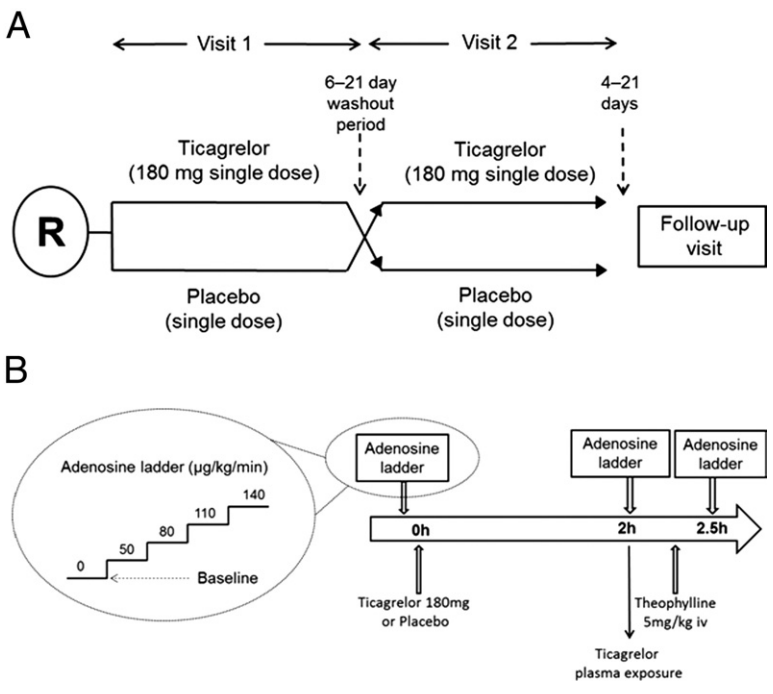

Figure 1 Study Design and Procedure

(A) Study design and **(B)** the procedures performed at visits 1 and 2. IV = intravenous; R = randomization.

AUC as a covariate; treatment, period, and sequence as fixed effects; and subject within sequence as a random effect.

We used mixed effects models similar to the aforementioned model to assess the effect of theophylline on the AUC of CBFV versus adenosine dose on ticagrelor and placebo (secondary endpoint), the differences in baseline blood flow (absence of adenosine) between ticagrelor and placebo before and after infusion of theophylline (secondary endpoint), and the effect of ticagrelor compared with placebo on the CBFV response at individual adenosine doses (exploratory endpoint).

Interday and intraday coefficients of variation (CV%) and SDs for the CBFV and Borg scale results were estimated by using a mixed-model analysis of variance with variance component estimations. Wilcoxon signed rank tests were used for testing the difference in Borg scale responses.

Results

CBFV response. The interindividual (predose) variation (CV%) for adenosine-induced CBFV-AUC was 0.268 and the intraindividual between-day (predose) and within-day (pre- vs. post-placebo) CV%s were 0.001 and 0.013, respectively (Figs. 2A and 2B).

The adenosine-induced CBFV-AUC increased 15% (95% confidence interval [CI]: 9 to 21) with ticagrelor versus 4% (95% CI: −1 to 10) for placebo ($p = 0.008$) (Fig. 3A). Assessing the effect of ticagrelor according to dose of adenosine, there was a significant increase in CBFV when adenosine was given at 50 and 80 μg/kg/min (95% CI: 0.1 to 10.0 and 6.7 to 29.1, respectively).

Theophylline infusion significantly reduced the adenosine-induced CBFV-AUC in both study groups (point estimates and 95% CIs: AUC-ticagrelor-theophylline/AUC-ticagrelor: 0.655 [0.612 to 0.702] vs. AUC-placebo-theophylline/AUC-placebo: 0.628 [0.586 to 0.673]). The reduction was not significantly different in subjects receiving ticagrelor or placebo (point estimate and 95% CI: 1.04 [0.946 to 1.15]; $p = 0.39$) (Fig. 3B).

CBFV in the absence of adenosine was similar before and after administration of ticagrelor (25.4 ± 6.1 cm/s vs. 26.3 ± 7.0 cm/s) and placebo (25.6 ± 6.3 cm/s vs. 24.9 ± 5.5 cm/s) and was not significantly attenuated by theophylline (25.4 ± 6.1 cm/s vs. 22.0 ± 5.5 cm/s and 25.6 ± 6.3 cm/s vs. 21.4 ± 5.0 cm/s for ticagrelor and placebo, respectively).

There was a significant correlation between ticagrelor, but not AR-C124910XX (data not shown), in terms of plasma concentrations and change in adenosine-induced CBFV-AUC ($r = 0.53$; $p < 0.001$) (Fig. 4).

Dyspnea. The interindividual (pre-dose) variation (SD) for adenosine-induced dyspnea was 0.90, and the intraindividual variability (SDs) between days (pre-dose) and within days (pre- vs. post-placebo) was 0.09 and 0.05, respectively.

Ticagrelor significantly augmented the adenosine-induced dyspnea at adenosine doses of 110 and 140 μg/kg/min ($p < 0.05$), whereas no difference was seen post-placebo (Fig. 5A). Comparing the Borg scale findings between the ticagrelor and placebo groups, values were significantly increased by ticagrelor at adenosine doses of 80, 110, and 140 μg/kg/min ($p < 0.01$).

Theophylline infusion significantly reduced the adenosine-induced dyspnea in both treatment groups, at all adenosine doses (except 50 μg/kg/min for placebo; $p < 0.01$) (Fig. 5B).

Safety. An imbalance of episodes of second-degree atrioventricular block was observed during adenosine infusion (ticagrelor: 6; placebo: 3). However, there was a similar imbalance during adenosine infusion predose (4 vs. 2). Post-theophylline, there was only 1 episode in the ticagrelor group versus none in the placebo group. All occurrences of atrioventricular block were of short duration and of no clinical significance.

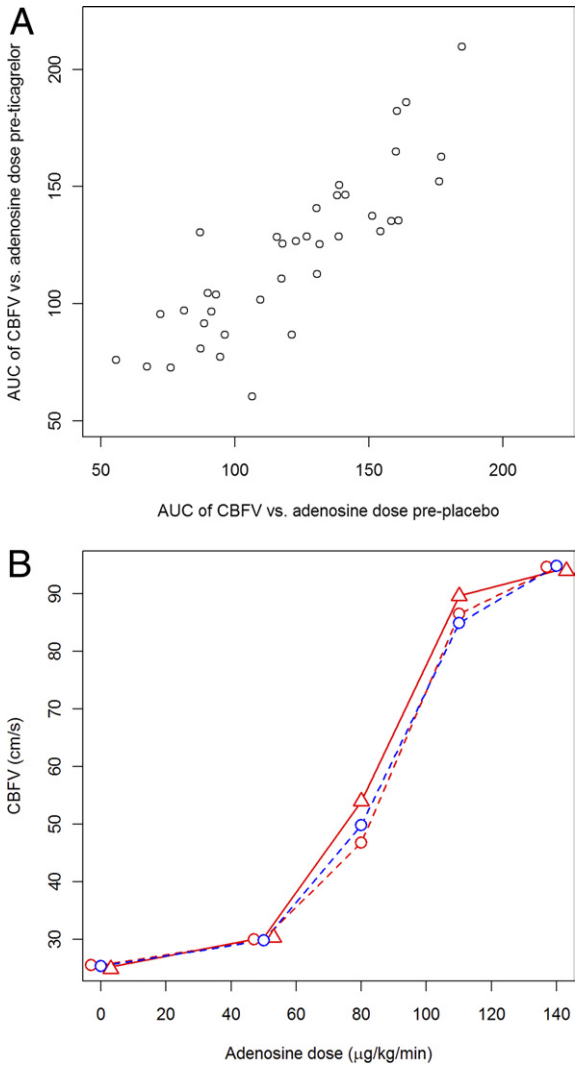

Figure 2 Variability and Reproducibility of the CBFV Measurement

(A) Adenosine dose versus mean coronary blood flow velocity (CBFV) response pre-placebo **(red dashed line, red circle), post-placebo (red solid line, red triangle)**, and pre-ticagrelor **(blue dashed line, blue circle)** and **(B)** the individual area under the curve (AUC) of the CBFV versus adenosine dose pre-placebo and pre-ticagrelor. Overlapping symbols have been slightly separated for visibility.

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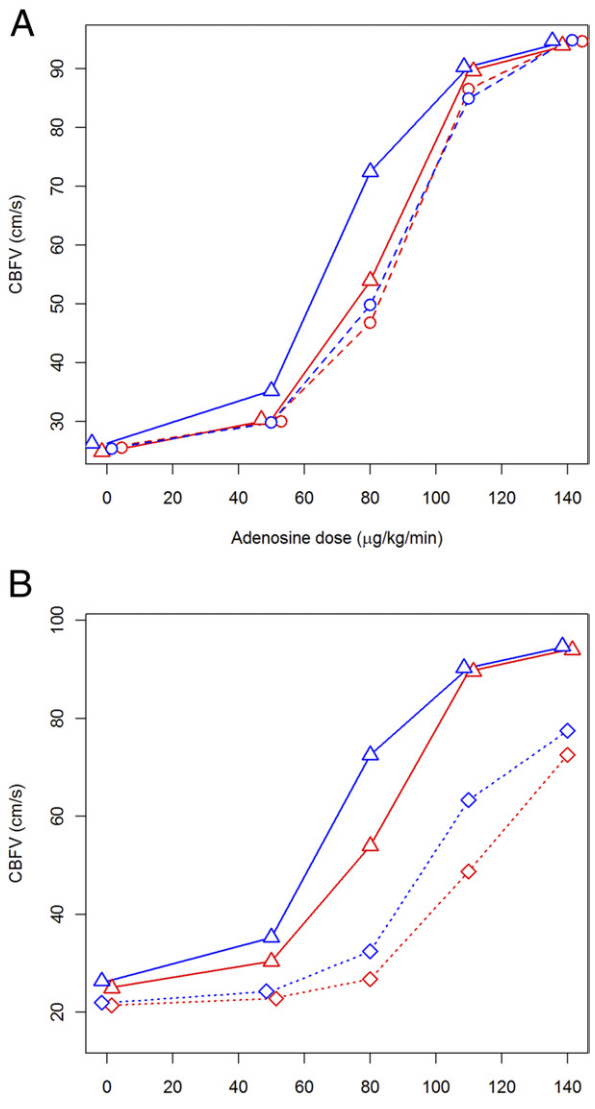


Figure 3 **Treatment Effect on Adenosine-Induced CBFV**

(A) Adenosine-induced mean coronary blood flow velocity (CBFV) and **(B)** mean CBFV after theophylline. Symbols are given by pre-placebo **(red dashed line, red circle)**, post-placebo **(red solid line, red triangle)**, pre-ticagrelor **(blue dashed line, blue circle)**, post-ticagrelor **(blue solid line, blue triangle)**, post–theophylline-placebo **(red dotted line, red diamond)**, and post–theophylline-ticagrelor **(blue dotted line, blue diamond)**. Overlapping symbols have been slightly separated for visibility.

Discussion

This study shows, for the first time, that ticagrelor augments adenosine-induced physiological responses in human subjects. Ticagrelor (180 mg) shifted the dose-response curve for adenosine-induced CBFV to the left (Fig. 3A) and significantly enhanced the AUC for CBFV response versus adenosine dose compared with placebo. The increase in AUC correlated with ticagrelor plasma concentrations. However, no correlation was found for the metabolite AR-C124910XX (approximately equipotent with ticagrelor on $P2Y_{12}$), suggesting that the parent compound is mainly driving the effect. The low interindividual and intraindividual variability over time for the CBFV response supports the hypothesis that differences in CBFV were a result of treatment effects and were not due to technical variability.

The enhanced vasodilation response to adenosine in the presence of ticagrelor was mediated by adenosine receptors, as demonstrated by a right shift of the adenosine dose-response curve after infusion of theophylline, a nonselective competitive adenosine receptor antagonist (Fig. 3B). The magnitude of the right shift of the adenosine dose-response curve induced by theophylline was similar for both placebo and ticagrelor. Thus, this study demonstrates an adenosine-related mode of action for ticagrelor in humans.

Ticagrelor, by preventing its cellular uptake, effectively extends the half-life of adenosine and will thereby increase extracellular levels of locally produced endogenous adenosine. This action may be important in vessel damage or hypoxia, where increased adenosine levels may contribute to vasodilation. In addition to promoting myocardial perfusion, there are additional adenosine-mediated cardioprotective effects that may be enhanced in patients treated with ticagrelor, such as ischemic preconditioning, inhibition of inflammatory response, and inhibition of platelet activation (8,9). In clinical trials, adenosine infusion has been shown to reduce myocardial infarct size but not major cardiovascular events (10,11). A possible advantage for ticagrelor over adenosine infusion is the local enhancement of adenosine levels in ischemic tissues (rather than a systemic effect).

Adenosine is known to induce dyspnea that is not associated with bronchospasm (4). In addition to augmentation of adenosine-induced CBFV, the current study dem-

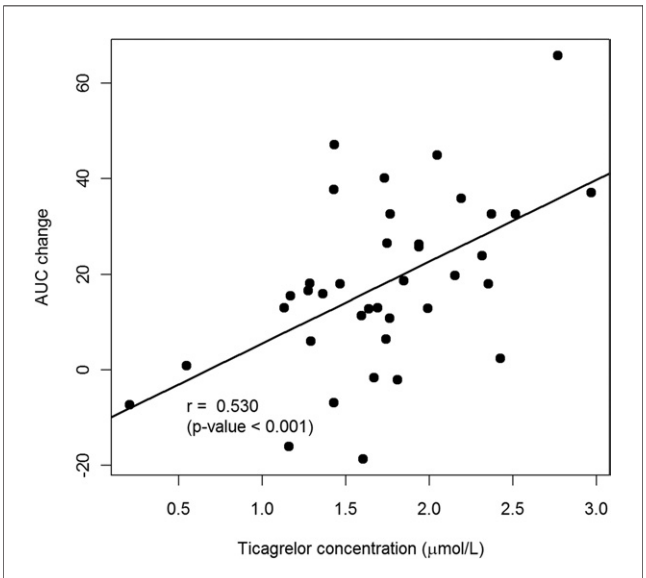
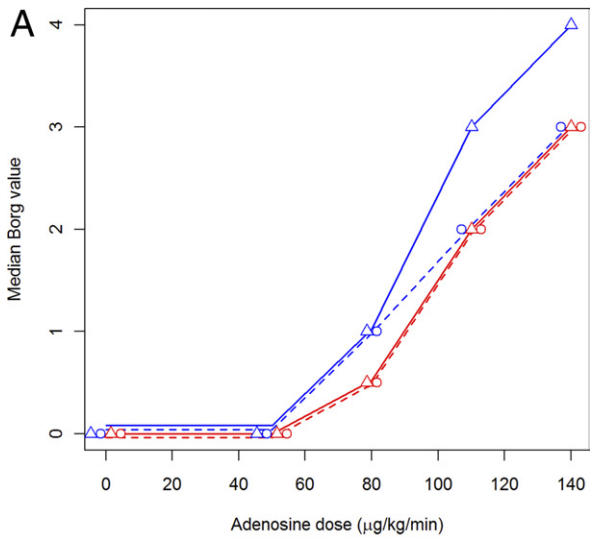


Figure 4 **Concentration-Dependent Effect by Ticagrelor on CBFV**

Correlation between individual plasma concentrations of ticagrelor and change in the AUC for the adenosine-induced CBFV response. Abbreviations as in Figure 2.



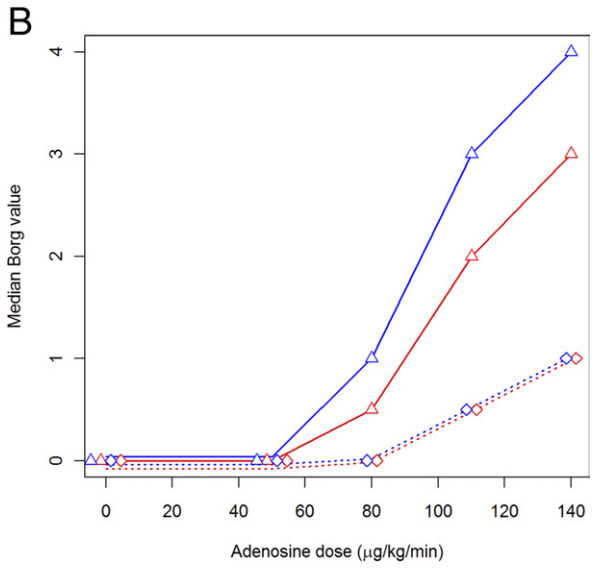


Figure 5 **Treatment Effect on Adenosine-Induced Dyspnea**

(A) Adenosine-induced median Borg scale and **(B)** the effect of the adenosine receptor antagonist theophylline. Symbols are given by pre-placebo **(red dashed line, red circle)**, post-placebo **(red solid line, red triangle)**, pre-ticagrelor **(blue dashed line, blue circle)**, post-ticagrelor **(blue solid line, blue triangle)**, post–theophylline-placebo **(red dotted line, red diamond)**, and post–theophylline-ticagrelor **(blue dotted line, blue diamond)**. Overlapping lines and symbols have been slightly separated for visibility.

onstrated that ticagrelor increased the sensation of adenosine-induced dyspnea according to the Borg scale. As with CBFV, the effect could be reversed by use of theophylline, an adenosine receptor antagonist. Thus, this study demonstrated for the first time that ticagrelor, at similar exposures as seen in the PLATO study (2), can augment adenosine-mediated biological responses in humans.

Taken together, this adenosine-mediated secondary mode of action of ticagrelor may provide a plausible mechanistic explanation for the cardioprotective effects, as well as the increased occurrence of dyspnea, observed in patients taking ticagrelor versus clopidogrel in the PLATO study (12). Further clinical studies are warranted.

Conclusions

This study was performed in healthy male volunteers after a single dose (180 mg) without co-medication and other confounding risk factors. The effects of exogenous (infused) rather than local endogenous adenosine were studied. Caution should be taken when extrapolating the findings to effects of chronic treatment in patients with acute coronary syndrome who may also have altered resting coronary blood flow dynamics due to advanced coronary artery diseases.

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Key Words: adenosine ■ coronary blood flow ■ dyspnea ■ ticagrelor.