# Adjunctive treatment with ticagrelor, but not clopidogrel, added to tPA enables sustained coronary artery recanalisation with recovery of myocardium perfusion in a canine coronary thrombosis model

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## Summary

Reperfusion therapy for myocardial infarction is limited by significant re-occlusion rates and less-than-optimal myocardial tissue perfusion. It was the objective of this study to assess and compare the effect of ticagrelor, the first reversibly binding oral $P2Y_{12}$ receptor antagonist, with that of clopidogrel, in conjunction with thrombolytic therapy, on platelet aggregation, thrombus formation, and myocardial perfusion in a canine model. Thrombus formation was induced by electrolytic injury and blood flow was measured with a Doppler ultrasonic flowmeter. All animals received tissue plasminogen activator (tPA) (1 mg/kg over 20 min); 10 animals received clopidogrel (10 mg/kg IV bolus over 5 min), 10 animals received ticagrelor initiated with a 1-min bolus (75 μg/kg/min), followed by continuous infusion (10 μg/kg/min) for 2 h, and 10 animals received IV saline. Re-occlusion rate and cyclic flow variation decreased with ticagrelor compared to saline groups ($p<0.05$). Adenosine phosphate (ADP)-induced platelet aggregation decreased with ticagrelor (1.9% ± 2.67) and clopidogrel (1.11% ± 2.0) vs. saline (26.3% ± 23.5, $p<0.05$) at the end of adjunctive therapy. Bleeding time increased in the clopidogrel compared to the ticagrelor group ($p=0.01$). Infarct size was reduced with ticagrelor compared to the clopidogrel and saline groups ($p<0.05$). Blood flow remained significantly below baseline values at 20 min after tPA administration in the saline and clopidogrel groups but not in the ticagrelor group. In conclusion, in a dog coronary thrombosis model, ticagrelor blocks ADP-induced platelet activation and aggregation; prevents platelet-mediated thrombosis; prolongs reperfusion time and reduces re-occlusion and cyclic flow variation; and significantly decreases infarct size and rapidly restores myocardial tissue perfusion.

## Keywords

$P2Y_{12}$ receptor, thrombosis, myocardial tissue perfusion, myocardial contrast echocardiography, ticagrelor

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## Introduction

Although considerable progress has been made in the treatment of ischaemic heart disease over the past decades, acute ischaemic complications due to excessive platelet accumulation, recruitment, and thrombus formation remain a challenging therapeutic issue. Adenosine diphosphate (ADP)-induced platelet activation plays a pivotal role in development of the thrombus via interaction with the $P2Y_1$ and $P2Y_{12}$ receptors (1), and thus inhibition of this pathway holds promise as a means of significantly reducing clot formation during acute ischaemic states (2). Currently, dual-antiplatelet therapy with aspirin and the thienopyridine clopidogrel, a $P2Y_{12}$ receptor antagonist, is standard treatment for patients with acute coronary syndromes (ACS) (3–5) and those undergoing percutaneous coronary intervention (PCI) (6, 7). However, many patients continue to have recurrent thrombotic events while receiving standard dual antiplatelet therapy (5). The effects of clopidogrel on platelets are slow and irreversible (8, 9), demonstrating modest antiplatelet effect with substantial interpatient variability (9, 10) and delayed onset of action (7).

Ticagrelor is the first reversibly binding oral $P2Y_{12}$ receptor antagonist. In contrast with the thienopyridines, this agent does not require metabolic conversion to an active form; binds directly to the $P2Y_{12}$ receptor; and can more completely inhibit the sustained platelet aggregation response to ADP (11). It has been suggested that ticagrelor has favourable pharmacokinetics and pharmacodynamics with less inter-individual variability of response (11, 12). A study in patients with stable atherosclerosis found a dose-dependent increase in the level of inhibition with ticagrelor, with levels being significantly higher at doses of 100 mg bid and above than those achieved with clopidogrel (11). The results from DISPERSE2 (Dose confIrmation Study assessing anti-Platelet Effects of ti-

cagrelor vs. clopidogrel in non-ST-segment Elevation myocardial infarction) trial showed that ticagrelor treatment was associated with a favourable trend toward lower risk of myocardial infarction (MI) and greater platelet aggregation inhibition in a dose-dependent fashion as compared with clopidogrel in both clopidogrel-experienced and clopidogrel-naive patients (13, 14). In the PLATO (PLATelet inhibition and patient Outcomes) trial of more than 18,000 ACS patients with or without ST-segment elevation, treatment with ticagrelor as compared with clopidogrel significantly reduced the rate of death from vascular causes, MI, or stroke, without an increase in the rate of overall major bleeding (15).

Microvascular dysfunction and reduced myocardial tissue perfusion often persist after patency of an infarct-related artery has been achieved with reperfusion therapy in acute MI. Antiplatelet therapy is frequently given adjunctively with thrombolytic therapy in acute MI. In this study, we investigated the effects of ticagrelor on reperfusion, re-occlusion, and infarct size when used adjunctively with thrombolytic therapy in a canine coronary thrombosis model and compared to clopidogrel in terms of reperfusion outcomes and hemorrhagic complications by assessing myocardial tissue blood flow using myocardial contrast echocardiography (MCE).

## Methods

### Surgical preparation

A canine coronary electrolytic injury thrombosis model was used as described previously (16, 17) using hound dogs (20–22 kg) of either sex. Briefly, following anesthesia with intravenous sodium pentobarbital (25 mg/kg) and left thoracotomy through the fifth intercostal space, a 2-cm segment of left circumflex coronary artery (LCX) was isolated, and a Doppler ultrasonic flow probe (Crystal Biotech, Northborough, MA, USA) was placed around the vessel for the measurement of coronary blood flow (CBF). CBF was monitored and recorded using a digital data acquisition system (PONEMAH Life Science Suite, Data Science International, St. Paul, MN, USA). Thrombosis was induced using the electrolytic injury technique (16). The endothelium of the LCX was injured by gently rubbing the artery distal to the flow probe. A coronary electrode, consisting of silver- hypodermic wire with a 26-gauge needle tip, was then inserted into the isolated and injured segment of LCX. Visual inspection of proper positioning of the electrode in the artery during the experiment was performed to ensure contact with the intraluminal surface of the vessel. The visual evidence of vessel wall injury surrounding the electrode was examined at the end of the experiment. Distal to the flow probe and the electrode, a vascular occluder was placed around the vessel and then adjusted to induce 80% stenosis. Thrombosis was initiated by delivery of 100-μA continuous anodal current to the tip of the coronary electrode, and formation of a fully occlusive coronary thrombus was determined by zero CBF. After thrombus formation, the occluder was gradually removed, and electric stimulation was then suspended. Throughout the procedure, electrocardiography was continuously monitored to determine heart rate and ST-segment elevation. Blood gas analysis was performed to maintain the pH and arterial blood gas within physiological range. All experiments were performed under the regulations of the Animal Welfare Act and the "Principles of Laboratory Animal Care" formulated by the Institute of Laboratory Animal Resources (National Research Council, NIH Publication No. 85–23, revised 1996) with the approval of the Animal Research Committee of the Cleveland Clinic. All animals involved in this study received humane care in compliance with act.

### Experimental protocol and drug administration

All animals had received aspirin (325 mg) orally the day of the procedure. After the occluder was removed and the electric stimulation was turned off, 30 minutes (min) were allowed to elapse to confirm thrombus stability prior to administration of tissue plasminogen activator (tPA, 1 mg/kg by 20 min infusion) (▶Fig. 1). At 25 min after thrombus formation (5 min prior to tPA), 10 animals received clopidogrel (10-mg/kg IV bolus over 5 min); 10 animals received ticagrelor initiated with a 1-min bolus (75 μg/kg/min), followed by continuous infusion (10 μg/kg/min) for 2 hours (h); and 10 animals received IV saline for 2 h. Simultaneously with administration of tPA, heparin was given as an 80-U/kg bolus IV followed by a continuous infusion of 17 U/kg/h for the subsequent duration of the experiment.

Thrombus lysis was defined as restoration of CBF to at least 30% of the baseline value, occurring at any time after the onset of drug infusion. Re-occlusion was defined as occurrence of zero CBF after successful thrombus lysis (16, 17). The animals were observed for a 2-h period after the occurrence of thrombus lysis for evidence of re-occlusion. If re-occlusion did not occur, the maximal duration of reperfusion was considered to be 120 min. In those animals with intermittent CBF restoration due to cyclic flow variations (CFVs), the total duration of reperfusion was calculated as the length of time during which CBF was greater than zero during the 2-h period after onset of reperfusion. Two hours after the onset of thrombus lysis, animals with persistent zero CBF as well as animals with CFVs were considered to have re-occlusion of the coronary artery. Xylocaine was administered as an IV bolus to control ventricular arrhythmia as necessary.

### Platelet aggregation

Peripheral venous blood samples were collected in 3.8% sodium citrate (9:1 v/v) at baseline, at the end of tPA infusion, and at the end of adjunctive treatment. Platelet-rich plasma (PRP) was obtained by centrifuging the blood at 150 g for 15 min at 24°C. Platelet-poor plasma (PPP) was obtained by further centrifugation at 1,500 g for 15 min at room temperature. Platelet counts in PRP were adjusted to $2.5 \times 10^8$ cells/ml. Final-extent aggregation was

 

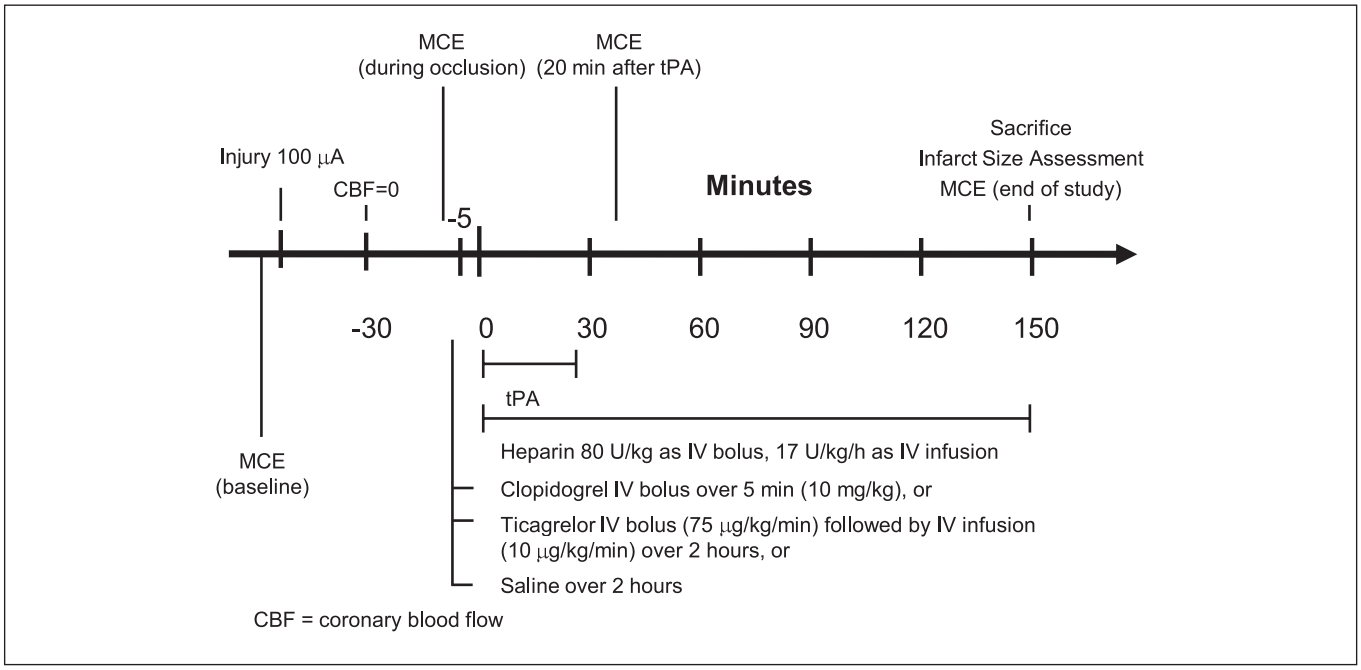


**Figure 1: Schematic diagram of experimental protocol.** Thrombus formation was induced by passage of a 100-μA continuous anodal current through the coronary electrode. At 30 min after thrombus formation, animals received tPA 1 mg/kg by 20-min infusion. Starting 5 mins prior to tPA, animals received IV saline infusion for 2 h (n=10), clopidogrel 10 mg/kg as a 5-min IV bolus (n=10), or ticagrelor as a 1-min bolus of 75 μg/kg/min followed by 10 μg/kg/min infusion for 2 h (n=10). MCE was performed at baseline, during the occlusion, 20 min after tPA infusion, and at the end of adjunctive treatment administration. All animals received heparin as an IV bolus (80 U/kg) followed by a continuous infusion (17 U/kg/h) for the subsequent period of the experiment. CBF, coronary blood flow; MCE, myocardial contrast echocardiography; tPA, tissue plasminogen activator.

determined with a light-transmission aggregometer (Chronolog Corp, Havertown, PA, USA) in response to ADP (20 μM).

## Coagulation study and bleeding time

Blood samples were taken at the same time points as platelet aggregation studies for measurement of prothrombin time (PT) and activated partial thromboplastin time (aPTT) using standard techniques with the ST4 Coagulation Timer (Diagnostica Stago, Parsippany, NJ, USA) (16, 17). Gum bleeding time (BT) was performed to determine the effect of ticagrelor on BT. Incision of the inner lip was carried out with a fully automated incision instrument (Surgicutt, International Technidyne Co, Edison, NJ, USA) (18). Every 30 seconds (s) after the incision was made, the flow of blood was wicked using blotting paper until it stopped.

## Determination of infarct size

At the end of the experiment, the animals were euthanised with overdose pentobarbital and potassium chloride; the heart was excised, and infarct size and area at risk were determined using the *ex vivo* dual perfusion histochemical method (16, 17). The LCX and left anterior descending coronary artery (LAD) were cannulated and perfused with 1.5% triphenyltetrazolium chloride (TTC) in the LCX and with Evans blue in the LAD simultaneously at the pressure of 100 mm Hg for 10 min at 37°C. After fixation with 10% formalin for 2 h, the heart was sectioned into five transverse slices, and the infarct area and the area at risk were quantified using a computerised image analyser system (Image-Pro Plus, Version 4.0 for Windows, Media Cybernetics, Silver Springs, MD, USA) on a blinded basis.

## Myocardial contrast echocardiography (MCE)

Real-time harmonic MCE was performed at baseline, during the occlusion, 20 min after tPA infusion, and at the end of the experiment. Previous experimental and clinical studies have shown that MCE is a reliable non-invasive method to quantify myocardial tissue perfusion (16, 19, 20). The heart was imaged in the short-axis view using GE Vingmed/Vivid FiVe. The echocardiographic contrast agent used in this study was Definity™ (DuPont Pharmaceuticals Co., Wilmington, DE, USA). One vial was diluted in 50 ml of normal saline solution, and the contrast was infused intravenously using a syringe pump at the rate of 4 ml/h. The area at risk (AR) was defined as delayed replenishment (1–2 s after burst) in the LCX territory. After full replenishment, the patchy area or the

**Table 1: Thrombolytic effects of tPA with heparin in animals treated with saline, clopidogrel, and ticagrelor.**

| | Saline (n=10) | Clopidogrel (n=10) | Ticagrelor (n=10) |
|---|---|---|---|
| Reperfusion rate, no. with reperfusion | 90% (9) | 100% (10) | 100% (10) |
| Time to reflow, minutes | 24.44 ± 7.74 | 27.22 ± 6.24 | 22.89 ± 8.10 |
| Reflow duration, minutes | 87.40 ± 44.92 | 97.90 ± 38.89 | 120.00 ± 0.00[1] |
| Cyclic flow variation rate, no. with cyclic flow variation | 30% (3) | 30% (3) | 0% (0) |
| Reocclusion rate, no. with reocclusion | 50% (5) | 30% (3) | 0% (0)[2] |

Data are mean ± SD. [1]p<0.05 compared to the saline group; there was no significant difference between the saline and clopidogrel group and no significant difference between the clopidogrel and ticagrelor groups (p =0.077). [2] p <0.05 compared to the saline group; there was no significant difference between the saline and clopidogrel group and no significant difference between the clopidogrel and ticagrelor groups.

**Table 2: PRP platelet aggregation (ADP 20 μM) in tPA-treated animals receiving saline, clopidogrel, or ticagrelor.**

| | % aggregation | | |
|---|---|---|---|
| | Saline (n=10) | Clopidogrel (n=10) | Ticagrelor (n=10) |
| Baseline | 36.1 ± 25.7 | 46.8 ± 25.0 | 31.1 ± 21.1 |
| End of tPA infusion | 31.3 ± 22.6 | 7.11 ± 6.5[1] | 7.33 ± 6.1[1] |
| 20 minutes after tPA | 35.5 ± 26.1 | 4.75 ± 4.13[1] | 6.56 ± 7.2[1] |
| End of experiment | 26.3 ± 23.5 | 1.11 ± 2.0[1] | 1.90 ± 2.67[1] |

Data are mean ± SD. [1] p <0.001 compared to saline group.

area without contrast was considered the infarct area. In order to determine myocardial perfusion at the tissue level, video intensity (dB, contrast signal intensity range)) was measured at different time points and fitted into an exponential model:

$$y = A(1 - e^{-bt}) + c,$$

where *A* was the plateau signal intensity reflecting the cross-sectional blood volume, *b* the rate of rise to the plateau reflecting myocardial blood velocity and *c* the intercept at the origin (21). The reperfusion rate (A/τ) was calculated at each time point. All data analysis was performed using software EchoPAC (Version 6.3.1) on a blinded basis.

### Statistical analysis

Statistical analysis was performed using SPSS software (Version 11.0 for Windows, SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). Perfusion data were analysed using Fisher's exact test. All other data were analysed using pairwise t-test with Bonferroni correction. A value of p≤0.05 is considered statistically significant.

**Table 3: Coagulation parameters in tPA-treated animals receiving saline, clopidogrel, or ticagrelor.**

| | PT, seconds | INR | aPTT, seconds |
|---|---|---|---|
| **Baseline** | | | |
| Saline | 9.12 ± 0.72 | 0.81 ± 0.71 | 15.8 ± 0.89 |
| Clopidogrel | 9.30 ± 0.62 | 0.83 ± 0.10 | 15.3 ± 0.73 |
| Ticagrelor | 8.96 ± 0.73 | 0.80 ± 0.10 | 15.7 ± 0.89 |
| P value | 0.582 | 0.656 | 0.436 |
| **End of tPA infusion** | | | |
| Saline | 9.53 ± 0.65 | 0.85 ± 0.10 | 32.44 ± 7.65 |
| Clopidogrel | 9.61 ± 0.71 | 0.86 ± 0.10 | 35.50 ± 8.00 |
| Ticagrelor | 9.32 ± 0.71 | 0.84 ± 0.10 | 31.00 ± 6.40 |
| P value | 0.723 | 0.786 | 0.519 |
| **20 minutes after tPA infusion** | | | |
| Saline | 9.63 ± 1.10 | 0.87 ± 0.13 | 30.50 ± 7.40 |
| Clopidogrel | 9.70 ± 0.82 | 0.87 ± 0.10 | 31.78 ± 6.86 |
| Ticagrelor | 9.28 ± 0.74 | 0.82 ± 0.10 | 28.92 ± 6.44 |
| P value | 0.656 | 0.570 | 0.752 |
| **End of experiment** | | | |
| Saline | 9.73 ± 0.79 | 0.88 ± 0.10 | 21.40 ± 3.40 |
| Clopidogrel | 10.00 ± 0.87 | 0.90 ± 0.10 | 46.50 ± 65.27 |
| Ticagrelor | 10.40 ± 3.10 | 0.97 ± 0.30 | 47.90 ± 64.77 |
| P value | 0.737 | 0.648 | 0.502 |

Data are mean ± SD. INR, international normalised ratio.

## Results

### Effects on thrombolysis and acute re-occlusion

The comparative thrombolytic effects in the three groups are shown in ▶Table 1. Baseline CBF was similar in all three groups. The mean time required for occlusive thrombus formation was also similar in the three groups (38.5 ± 21.6 min in the ticagrelor group vs. 41.1 ± 16.1 min in the clopidogrel group and 39.6 ± 23.6 min in the saline group, p=NS). Reperfusion occurred in each of the 10 animals in the clopidogrel and ticagrelor groups and in nine of the 10 saline group, and there were no differences among groups with regard to time to reflow. No CFV or re-occlusion occurred in any ticagrelor-treated animals, compared with CFV and re-occlusion in three and five animals, respectively, in the saline group (p<0.05 when compared to ticagrelor group) and in three and three animals, respectively, in the clopidogrel group (p=NS when compared to either ticagrelor or saline group). The mean duration of reflow in the ticagrelor group, reflecting the absence of CFV and

re-occlusion, was 120 min, significantly longer than that in the saline group. There is a trend of longer duration of reflow in the ticagrelor group compared to that in the clopidogrel group (p=0.077); there was no significant difference between the clopidogrel group and the saline group in duration of reflow.

### Platelet aggregation, coagulation study and bleeding time

The results of PRP platelet aggregation in response to ADP are summarised in ▶Table 2. tPA alone had no influence on platelet aggregation. The p-value between ticagrelor and saline groups at baseline is 0.648. The p-value between ticagrelor and clopidogrel groups at the end of the experiment is 0.464. However, adjunctive administration of ticagrelor or clopidogrel significantly decreased platelet aggregation when compared to saline group.

After administration of tPA, aPTT values increased in all groups, with no between-group differences (▶Table 3). There were no differences among groups in PT or aPTT at the end of the experiment. Bleeding time was significantly prolonged with clopidogrel and ticagrelor compared with saline throughout the experiment (▶Table 4). Bleeding time was non-significantly shorter with ticagrelor vs. clopidogrel at 20 min after tPA infusion and significantly shorter (5.16 vs. 8.24 min, p<0.05) by the end of the experiment.

**Table 4: Gum bleeding time in tPA-treated animals receiving saline, clopidogrel, or ticagrelor.**

| | Bleeding time, minutes | | |
|---|---|---|---|
| | Saline | Clopidogrel | Ticagrelor |
| Baseline | 1.26 ± 0.46 | 1.01 ± 0.36 | 1.09 ± 0.32 |
| End of tPA | 6.59 ± 3.32 | 10.00 ± 0.00[1] | 10.00 ± 0.00[1] |
| 20 minutes after tPA | 5.28 ± 2.60 | 9.40 ± 1.20[1] | 8.72 ± 1.79[1] |
| End of experiment | 2.23 ± 0.67 | 8.24 ± 2.61[1] | 5.16 ± 2.17[1,2] |

Data are mean ± SD. [1] p <0.001 compared to saline group. [2] p <0.05 compared to clopidogrel group.

**Table 5: Myocardial area at risk and infarct size in tPA-treated animals receiving saline, clopidogrel, or ticagrelor.**

| | Saline (n=10) | Clopidogrel (n=10) | Ticagrelor (n=10) |
|---|---|---|---|
| Area at risk ($cm^2$) | 64.19 ± 7.89 | 60.20 ± 7.90 | 60.91 ± 6.92 |
| Infarct size ($cm^2$) | 13.63 ± 4.19 | 14.63 ± 4.29 | 6.31 ± 2.86[1] |
| Infarct size as % of area at risk (%) | 28.00 ± 9.21 | 32.66 ± 10.14 | 11.70 ± 5.55[1] |

Data are mean ± SD. [1] p <.05 compared to both the saline group and the clopidogrel group.

### Infarct size, myocardial contrast echocardiography

Compared to saline and clopidogrel groups, infarct size was significantly reduced, by more than 50%, in the ticagrelor group whether expressed as percentage of the area at risk or as absolute infarct size (absolute infarct size: 14.63 ± 4.29 $cm^2$ in clopidogrel group vs. 6.31 ± 2.86 $cm^2$ in ticagrelor group, p<0.001; infarct size as % of area at risk: 32.66 ± 10.14% in clopidogrel group vs. 11.70 ± 5.55% in ticagrelor group, p<0.001) (▶Table 5).

Myocardial tissue blood flow was significantly decreased in all three groups during occlusion and increased in all groups by 20 min after tPA (▶Table 6, ▶Fig. 2). There were no significant differences among the three groups with regard to blood flow at baseline. However, by 20 min after tPA, blood flow remained significantly lower than baseline levels in the saline and clopidogrel groups, whereas that in the ticagrelor group no longer differed significantly from baseline. Similarly, at the end of the experiment, the perfusion rate in the ticagrelor group trended higher than that in the other groups, with the reduction from baseline in the saline (p=0.060) and clopidogrel (p=0.051) groups. However, there is no significant difference found among three groups at the end of the experiment (ticagrelor vs. saline, p=0.497; ticagrelor vs. clopidogrel, p=0.254, clopidogrel vs. saline, p=0.618).

## Discussion

ADP is a key mediator of platelet aggregation and thrombosis, particularly under the high-shear flow conditions characteristic of stenosed atherosclerotic arteries. In this study, we demonstrated that

**Table 6: Myocardial perfusion rate assessed by myocardial contrast echocardiography (MCE) in tPA-treated animals receiving saline, clopidogrel, or ticagrelor.**

| | Myocardial perfusion rate (A/τ) (dB/sec) | | | |
|---|---|---|---|---|
| | Baseline | During occlusion | 20 minutes after tPA | End of experiment |
| Saline | 3.741 ± 1.431 | 0.843 ± 0.573[1] | 2.425 ± 0.900[1] | 2.320 ± 1.728[2] |
| Clopidogrel | 3.768 ± 1.987 | 1.305 ± 1.050[1] | 1.835 ± 1.280[1] | 1.894 ± 2.017[3] |
| Ticagrelor | 3.846 ± 2.192 | 1.203 ± 0.840[1] | 2.694 ± 1.102 | 2.806 ± 1.390[4] |

Data are mean ± SD. [1] p <0.05 compared to baseline. [2] p =0.060 compared to baseline. [3] p =0.051 compared to baseline. [4] p =0.221 compared to baseline.

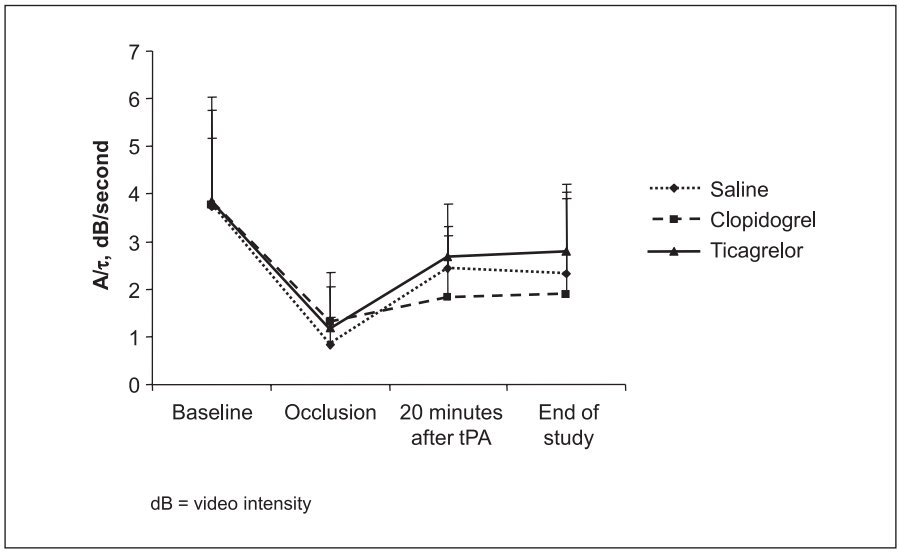


**Figure 2: Myocardial microvascular flow assessed by myocardial contrast echocardiography (MCE) at baseline, during occlusion, 20 min after tPA infusion, and at the end of adjunctive treatment administration.** There was no significant difference in myocardial tissue blood flow among the three groups at these time points. At 20 min after tPA, tissue blood flow remained significantly lower than baseline levels in the saline and clopidogrel groups but not in the ticagrelor group. dB = video intensity.

inhibition of ADP-mediated platelet aggregation and recruitment by the $P2Y_{12}$ receptor antagonist ticagrelor significantly prolongs reperfusion time and abolishes re-occlusion and CFV in a canine coronary thrombosis model compared with tPA alone. Ticagrelor treatment was associated with a rapid return of myocardial tissue perfusion towards the baseline rate and resulted in a significant reduction in infarct size compared with clopidogrel and tPA alone. While exerting these effects, ticagrelor had less bleeding time increase than clopidogrel.

Arterial thrombi that are rich in platelets are relatively resistant to fibrinolysis and prone to induce re-occlusion after initial reperfusion (22). Despite the inhibition of cyclooxygenase by aspirin, platelet activation can still occur through thromboxane $A_2$-independent pathways, leading to the aggregation of platelets and the formation of thrombin (23). Adenine nucleotides affect a number of cellular events. Activation of platelets by ADP is a receptor-mediated process involving two subtypes of the P2 receptor family: the $P2Y_1$ and-$P2Y_{12}$ receptors (24). It has been demonstrated that $P2Y_{12}$ receptor activation is important for the sustained platelet aggregation response to ADP and for a significant component of the platelet response to other agonists (25, 26). These findings were further confirmed by the findings that the platelets in a patient lacking the $P2Y_{12}$ receptor showed impaired ADP-dependent platelet aggregation, had much-reduced ADP-binding capacity, and lacked the ability to inhibit cyclic-GMP levels in response to ADP (27–29).

The importance of this receptor pathway in arterial thrombosis is also suggested by the positive clinical findings with ticlopidine and clopidogrel (3, 30), which produce partial inhibition of $P2Y_{12}$-mediated platelet activation following hepatic generation of an active metabolite (31). Clopidogrel is a widely used antiplatelet agent that has a synergistic antithrombotic effect when combined with aspirin (32). Dual therapy with aspirin and clopidogrel has become standard treatment in patients with ACS with or without ST-segment elevation and after stent procedures (3, 5, 7, 33). Clopidogrel has been shown to benefit patients with documented atherosclerosis (recent MI, recent stroke, or established peripheral arterial disease), patients who have undergone PCI, and patients with unstable angina or MI.

Despite these benefits, important limitations of clopidogrel remain, including a modest inhibition of platelet aggregation (34–38), substantial inter-patient variability in response (10, 38–39), and delayed onset of action associated with two-step metabolic activation and variable metabolic conversion to the active metabolite (7, 35, 37, 38). Small clinical studies have suggested that patients with a reduced pharmacologic response to clopidogrel may be at increased risk for adverse clinical events, including MI and coronary stent thrombosis (37, 40–42). Some of the limitations of clopidogrel have been overcome by the new thienopyridine, prasugrel, which is more efficiently metabolised and exhibits faster, greater, and more consistent platelet inhibition (37). Recently, prasugrel treatment was shown to reduce risk of ischaemic events compared with clopidogrel in PCI-treated ACS patients

### What is known about this topic?

- Platelet aggregation at sites of vascular injury contributes to limitations of reperfusion therapy following myocardial infarction.
- Ticagrelor, the first reversibly binding oral $P2Y_{12}$ receptor antagonist, results in higher and more consistent platelet inhibition the clopidogrel.

### What does this paper add?

- When administered in combination with thrombolytic therapy, ticagrelor results in prolonged reperfusion times and reductions in reocclusion and cyclic flow variation in canine models of coronary thrombosis.
- Combination therapy also significantly decreased infarct size and rapidly restored myocardial tissue perfusion, a finding that holds promise for patients requiring rapid restoration of blood flow following an incidence of myocardial infarction.

(43), but treatment with the agent, which like other thienopyridines is an irreversible $P2Y_{12}$ receptor antagonist, was also associated with a significantly increased risk of major bleeding, including life-threatening and fatal bleeding.

The irreversible binding of clopidogrel results in slow offset of effect, with gradual recovery of platelet function after drug withdrawal that is dependent on the generation of fresh platelets (44). To avoid an increased risk for serious bleeding, an interval of 5–7 days off clopidogrel in patients undergoing coronary artery bypass grafting is recommended (45, 46). Thus, the development of $P2Y_{12}$ receptor antagonists that are reversible and that exhibit a better balance between efficacy and safety is desirable. Ticagrelor is the first of the new cyclopentyl-triazolo-pyrimidine class of antiplatelet agents (11). Like the thienopyridines, ticagrelor blocks the platelet $P2Y_{12}$ receptor to inhibit ADP's prothrombotic effects. Unlike the thienopyridines, ticagrelor is orally active without the requirement for metabolic activation and binds reversibly to the $P2Y_{12}$ receptor, nearly completely inhibiting ADP-induced platelet aggregation *ex vivo*. Ticagrelor has one known active metabolite that is present in blood at about one third the concentration of the parent compound in studies in healthy volunteers (47). This metabolite is approximately as potent as ticagrelor at blocking the $P2Y_{12}$ receptor *in vitro* and is thought to contribute to antiplatelet effects after oral dosing with ticagrelor. The pharmacokinetic/pharmacodynamic profile of ticagrelor is characterized by rapid attainment of steady-state plasma concentrations, occurring by 2 h post-dose on the first day of dosing and low inter-individual variability of platelet inhibitory response (11); the agent's half-life is 12 h, with the antiplatelet effect being substantially reduced by 48 h after the last dose (12, 13). This reversibility might offer greater flexibility for surgical procedures. The phase III PLATO study (15) demonstrated that treatment with ticagrelor resulted in a lower risk of recurrent thrombotic events compared with clopidogrel in a broad ACS patient population without an increase in the rate of overall major bleeding.

Increasing evidence suggests that even when patency of an infarct-related artery has been reestablished, disordered microvascular function and inadequate myocardial tissue perfusion are often present following reperfusion therapy for acute MI. Thus, optimal reperfusion should not only sustain epicardial patency but also restore microvascular flow and myocardial tissue perfusion. An important finding of this study is that the combination of thrombolytic treatment and ticagrelor not only blocks ADP-induced platelet aggregation, prolongs reperfusion time, and abolishes re-occlusion but also significantly reduces infarct size and rapidly restores myocardial tissue perfusion as evidenced by MCE. These findings with ticagrelor are consistent with those in our previous study of AR-C69931MX (16), an intravenous agent that is a similar highly potent, reversible, and selective $P2Y_{12}$ receptor antagonist. A potential explanation for these findings is that the presence of a potent antiplatelet agent such as ticagrelor in the systemic circulation may relieve microvascular obstruction caused by platelet-thrombin microemboli; in contrast, the binding of clopidogrel to platelets appears to occur largely in the liver, limiting effects in the systemic circulation (35, 48). A further consequence of the systemic exposure of reversible antagonists could be their ability to affect $P2Y_{12}$ expressed on vascular smooth muscle cells as described by Wihlborg et al. (49). However, there is no evidence yet for a role of this receptor in *in vivo* models.

Moreover, studies characterising the GPR17 receptor, a close relative of both P2Y and cysLT (cysteinyl-leukotriene) receptors that is highly expressed in organs undergoing ischaemic damage (brain, heart, kidney) and that is dually activated by uracil nucleotides and cysLTs, showed that cangrelor (AR-C69931MX) antagonised GPR17 *in vitro* and reduced brain infarct size in a rat stroke model (50). Ticagrelor was also found to counteract GPR17 activation *in vitro*; in addition, it acted as a partial GPR17 agonist in the absence of nucleotides or cysLTs, suggesting that it may have neuroprotective effects during acute ischaemia while preserving GPR17 actions under normal conditions (51).

Another potential explanation for the effects of ticagrelor in the current study is the finding that ticagrelor potently inhibits adenosine uptake by erythrocytes, likely via inhibition of ENT-1 (52). In an anaesthetised dog model of CBF regulation, the prolongation of adenosine half-life *in vivo* by ticagrelor resulted in increased adenosine-induced reactive hyperaemia and CBF, suggesting activity and benefit in restoring flow beyond that achieved by inhibition of platelet aggregation (52). Ticagrelor's chemical structure itself does not feature any purine-like features and neither do its metabolic products (47) and a direct effect on adenosine receptors has not been found (AstraZeneca R&D Mölndal, data on file). Interestingly, recent data using a murine model of ischaemia-reperfusion injury in ENT-1 knock-out mice suggests that inhibition of ENT-1 may result in cardioprotective effects (53). Taken together, it is not unthinkable that some of the additional benefit of the reversible $P2Y_{12}$ antagonist ticagrelor seen in this study may result from effects on either vascular $P2Y_{12}$ or other non-$P2Y_{12}$ related targets.

One limitation of this study is that ACT was not measured since heparin was administered. However, we would not have expected to observe any differences in measurements of ACT among the three groups since all animals from this study received heparin.

## Conclusion

Ticagrelor is a novel, potent and reversible inhibitor of ADP-induced platelet aggregation through its $P2Y_{12}$ receptor antagonistic property. The administration of ticagrelor in the canine coronary thrombosis model blocks ADP-induced platelet aggregation and recruitment and prevents platelet-mediated thrombosis. It prolongs reperfusion time, abolishes re-occlusion and cyclic flow variations, significantly decreases infarct size, and restores myocardial tissue perfusion with only a modest increase in bleeding time.

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