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Full Length Article

Effect of $P2Y_{12}$ inhibitors on thrombus stability and endogenous fibrinolysis

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ABSTRACT

Although used routinely to reduce thrombotic events in patients with coronary disease, the effects of $P2Y_{12}$ inhibitors on thrombus stability and endogenous fibrinolysis are largely unknown.

Blood taken from patients pre- and post-aspirin (n = 20) and on aspirin alone and on dual antiplatelet therapy comprising aspirin plus clopidogrel (n = 20), ticagrelor (n = 20) or cangrelor (n = 20), was tested using the Global Thrombosis Test. The number of "rebleeds" or drops (D) after early platelet-rich thrombus formation (occlusion time, OT), and before final lasting occlusion, was used as an inverse measure of thrombus stability. Whilst clopidogrel had no effect, ticagrelor and cangrelor both increased D significantly, reflecting increased thrombus instability [D pre- and post-clopidogrel 4.3 ± 1.6 vs. 4.5 ± 1.4, p = 0.833; pre- and post-ticagrelor 4.1 ± 2.4 vs. 6.8 ± 5.1, p = 0.048; pre- and post-cangrelor 3.6 ± 2.0 vs. 7.9 ± 8.9, p = 0.046]. Platelet reactivity was reduced by all $P2Y_{12}$ inhibitors, demonstrated by OT prolongation (clopidogrel 378 ± 87 s vs. 491 ± 93 s, p < 0.001; ticagrelor 416 ± 122 s vs. 549 ± 121 s, p < 0.001; cangrelor 381 ± 146 s vs. 613 ± 210 s, p < 0.001). The magnitude of OT prolongation compared to baseline (ΔOT) was significantly greater for cangrelor compared to clopidogrel and ticagrelor. Cangrelor was the only agent to enhance fibrinolysis (lysis time pre- and post-cangrelor 1622[1240–2048]s vs. 1388[960–1634]s, p = 0.005).

We demonstrate the ability to assess the effect of pharmacotherapy on thrombus stability in vitro and show that $P2Y_{12}$ inhibitors potentiate thrombus instability at high shear. Cangrelor, and to a lesser extent ticagrelor, de-stabilised thrombus formation and cangrelor also enhanced fibrinolysis. Potentiation of thrombus instability could become a new pharmacological target, that may be particularly important in acute coronary syndromes.

1. Introduction

The stability of a developing arterial thrombus, namely its ability to cause lasting vessel occlusion, withstanding embolization and fibrinolysis, is a major determinant of subsequent downstream tissue damage [1,2]. A stable thrombus comprised of a platelet-rich core, held together by tight platelet-platelet contacts with a dense fibrin network, may have sufficient stability to resist high shear arterial flow conditions, resulting in long-lasting vessel occlusion. In comparison, unstable thrombi, that have sparse platelet-platelet contacts and less dense fibrin mesh, will be more susceptible to the effects of fibrinolytic proteins and dislodgement by arterial flow. This results in maintained vessel patency and a shower of macro- or micro-emboli, whose susceptibility to lyse in the circulation will determine downstream clinical sequelae.

Compared to the extensive investigation of the crucial role of platelets, coagulation and flow in arterial thrombosis, little attention has been paid to factors affecting thrombus stability. This is at least in part due to the previous lack of techniques available, other than cumbersome experimental laboratory models, mainly in animals, and the need to use non-anticoagulated blood. One of the challenges of assessing the effects of pharmacotherapy on fibrinolysis and thrombus stability, is that the thrombus formed in vitro must be allowed to become "stable", and the key driver imparting stability to the thrombus and resistance to lysis, is thrombin. Since thrombin generation is inhibited by the most commonly used anticoagulant, citrate, the contribution of thrombin to stability or resistance to fibrinolysis cannot be assessed when using anticoagulated blood [1].

Platelet activation and aggregation play a pivotal role in coronary

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease; GTT, Global Thrombosis Test; IPA, inhibition of platelet aggregation; LT, lysis time; OT, occlusion time; PCI, percutaneous coronary intervention; STEMI, ST-elevation myocardial infarction

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atherothrombosis. $P2Y_{12}$ inhibitors are routinely used as an adjunct to aspirin, to prevent or treat arterial thrombosis in patients with established coronary artery disease (CAD) in the setting of percutaneous coronary intervention (PCI) or acute coronary syndromes (ACS) [3–5]. The efficacy of many antithrombotic agents is shear-dependent and suggests that such agents may act by increasing the fragility of the growing thrombus or enhancing endogenous fibrinolytic activity. Currently available antiplatelet medications inhibit thrombus formation by preventing platelet activation, and thereby aggregation, in response to specific agonists, but the in vivo antithrombotic effects, including the specific effects on thrombus stability and endogenous fibrinolysis, are largely unknown.

Although differences between $P2Y_{12}$ inhibitors with respect to their impact on thrombus stability and fibrinolysis may not be of much clinical significance in stable CAD, where the incidence of thrombotic events is low, such effects may be important in the setting of ACS and in particular ST-elevation myocardial infarction (STEMI).

We therefore sought to assess and compare the effects of three different $P2Y_{12}$ inhibitors on thrombus stability and endogenous fibrinolysis.

2. Methods

2.1. Study design and population

This single centre, prospective observational study was approved by the national research ethics service and all patients gave written informed consent. Patients with inducible ischaemia documented by functional imaging and scheduled to undergo elective PCI for single vessel disease were recruited. Blood samples were taken to assess in vitro thrombotic status, when patients were taking aspirin as sole antiplatelet therapy alone, as well as after being established on additional $P2Y_{12}$ inhibitor ($n = 60$). Another separate group of patients with newly diagnosed stable angina also were recruited and blood drawn to assess thrombotic status before and after being established on aspirin ($n = 20$). Exclusion criteria were as follows: age < 18 years, patients unable to provide consent, those with atrial fibrillation, active sepsis or infective illness within the last month, known active malignancy, bleeding diathesis, blood dyscrasia (platelets $< 100 \times 10^9$/L, haemoglobin < 80 g/L, international normalized ratio [INR] > 1.4, activated partial thromboplastin time [aPTT] more than twice the upper limit of normal, leukocyte count $< 3.5 \times 10^9$/L, neutrophil count $< 1 \times 10^9$/L), the use of anticoagulation or antiplatelet therapy (other than aspirin) at the time of initial sampling or within the last month, and alcohol consumption above 14 units per week.

2.2. Aspirin and $P2Y_{12}$ inhibitor treatment

The choice of aspirin and $P2Y_{12}$ inhibitor was decided by the clinical team looking after the patient. Patients ($n = 20$) were tested before and 7–10 days after aspirin 300 mg oral loading followed by 75 mg once daily. A further 60 patients were tested after at least a week of aspirin treatment as above (baseline) and after being established on treatment with a $P2Y_{12}$ inhibitor comprising of clopidogrel ($n = 20$), ticagrelor (n = 20) or cangrelor (n = 20). Patients were treated with one of the following regimens prior to PCI: clopidogrel 300 mg oral loading followed by 75 mg once daily for seven days before PCI, ticagrelor 180 mg oral loading followed by 90 mg twice daily for seven days before PCI, or cangrelor 30 μg/kg intravenous bolus followed by a 4 μg/kg/min continuous intravenous infusion on the day of the procedure, prior to the administration of any procedural heparin anticoagulation. Patients were reminded of the need for compliance 7 days before each blood draw and compliance was confirmed by asking the patient at each visit.

2.3. Blood sampling

Blood samples were taken on two occasions from an antecubital vein: [1] at the baseline visit, and [2] on treatment, which consisted of either aspirin 75 mg daily monotherapy ($n = 20$) or dual antiplatelet therapy with aspirin and $P2Y_{12}$ inhibitor orally for 7 days or on intravenous cangrelor infusion for at least 10 min, after loading. Blood samples were taken approximately 3 h after the last oral dose or after 10 min of continuous maintenance cangrelor infusion. Fasting was not required. All samples were collected from an antecubital vein using an 18-G butterfly cannula using a two-syringe technique. The blood samples on $P2Y_{12}$ inhibitor treatment were always taken before heparin or bivalirudin administration and before PCI was performed. The first 5 mL was used for standard blood tests (biochemistry, full blood count) and the second 5 mL used for assessment. The second sample was inserted into the Global Thrombosis Test (GTT) for analysis within 15 s of withdrawal as described previously [6].

2.4. Assessment of thrombotic status

Thrombotic status was assessed using the point-of-care Global Thrombosis Test (GTT, Thromboquest Ltd., London, UK). This fully automated test assesses both platelet reactivity to high shear stress and endogenous fibrinolysis. The test is performed on 4 mL of native, non-anticoagulated blood which, immediately after withdrawal, is introduced into one of the 4 ports in the instrument, and thereafter the measurement is fully automated. The principle and the technique of the GTT have previously been described in detail [6].In brief, blood flows under the influence of gravity in a conical tube through two narrow apertures where it is subjected to high shear. This induces platelet activation, culminating in thrombus formation and thrombotic occlusion, resulting in slowing and eventual arrest of flow. A sensor detects flow downstream, and as the thrombus starts to form, with gradual reduction in flow, the time interval between consecutive blood drops increases. When the interval between drops exceeds 15 s, this is recorded as the occlusion time (OT) in seconds. In the second phase of the test, the instrument measures the lysis time (LT) in seconds, namely the time required to spontaneously dissolve the thrombus formed in the first phase, through endogenous fibrinolysis. The pre-set cut-off time for the OT is 900 s and for the LT is 6000 s. The intra-assay and inter-assay coefficients of variation (CV) for OT and LT were assessed on 10 subjects on repeated sampling and running samples in parallel.

2.5. Assessment of thrombus stability

As the thrombus builds up inside the narrow gaps in the GTT conical tube, downstream flow is reduced and eventually stops altogether. The gradual reduction in flow as the thrombus forms, is reflected in greater intervals between consecutive blood drops detected downstream by the photosensor. When the time interval between consecutive blood drops reaches 15 s or more, the instrument records this as the occlusion time (OT). However, this is the time taken to form early thrombus, that is fragile and unstable and may dislodge in part, and reform, before final arrest of flow (complete occlusion). The number of “rebleeds” or drops seen after OT, and before final lasting occlusion is a manifestation of thrombus stability, with more drops reflecting more instability. A feature of the newer GTT-3 model is that the software allows the visualisation of a graphic representation of the number of drops (D) from beginning of occlusion (OT) to complete occlusion, which was recorded as an inverse measure of stability in this study.

2.6. Statistical analysis

This was an exploratory study and patients acted as self-controls. A sample of 20 patients per group has been previously shown to be sufficient to give representative results for self-control groups [7,8]. Data

Table 1
Baseline Patient Characteristics.

	Aspirin (n = 20)	Clopidogrel (n = 20)	Ticagrelor (n = 20)	Cangrelor (n = 20)	P value
Age, yrs	60 ± 9	66 ± 11	65 ± 8	64 ± 11	0.383
Male	13(65)	16(80)	12(60)	15(75)	0.497
BMI *	28[27–32]	24.6[23–27]	26.3[22 – 30]	24.8[24–28]	0.07
Current smoking	3(15)	4(20)	3(15)	3(15)	0.965
Diabetes mellitus	6(30)	4(20)	4(20)	1(5)	0.242
Hypertension	10(50)	13(65)	12(60)	10(50)	0.712
Chronic kidney disease (eGFR < 60)	0(0)	1(5)	0(0)	1(5)	0.562
Prior MI	0(0)	5(25)	4(20)	2(10)	0.101
Prior PCI	0(0)	4(20)	3(15)	2(10)	0.223
Prior CABG	0(0)	1(5)	1(5)	0(0)	0.562
Left ventricle function					
Normal (EF ≥ 55%)	18(90)	16(80)	17(85)	17(85)	0.789
Mildly impaired (EF 45–54%)	1(5)	2(10)	2(10)	1(5)	0.586
Moderately impaired (EF 36–44%)	1(5)	2(10)	1(5)	1(5)	0.868
Severely impaired (EF ≤ 35%)	0(0)	0(0)	0(0)	1(5)	0.369
Concomitant medications					
Aspirin	0(0)	20(100)	20(100)	20(100)	< 0.001
Beta-blocker	7(35)	13(65)	11(55)	7(35)	0.144
Calcium antagonist	5(25)	3(15)	6(30)	5(25)	0.726
ACE inhibitor	8(40)	11(55)	10(50)	7(35)	0.568
Statin	13(65)	17(85)	15(75)	11(55)	0.190
Proton pump inhibitor	4(20)	6(30)	3(15)	3(15)	0.599
Blood tests on initial sampling					
Haemoglobin (g/L)	143[138–149]	142[128–150]	140[128–145]	144[130–151]	0.709
Haematocrit (%)	42[39–43]	40[38–44]	42[38–44]	43[39–45]	0.890
Platelet count ($\times 10^9$/L) *	245[220–293]	222[196–282]	239[188–283]	217[186–252]	0.226
Creatinine (μmol/L) *	82[68–90]	88[74–99]	87[78–97]	89[77–104]	0.125
C-reactive protein (mg/L)	1.0[1.0–3.0]	2.0[1.0–3.0]	3.0[2.0–3.5]	3.0[2.0–3.0]	0.206
Thrombotic status Baseline OT (*sec*)	431 ± 104	378 ± 87 s	416 ± 122 s	381 ± 146 s	0.149
Baseline LT (sec) *	1920[1730–2481]s	1826[1320–2279]s	1522[1347–1915]s	1622[1240–2048]s	0.084

Values are mean (standard deviation) or n (%), except * where values are median[IQR]. Left ventricular function was assessed by a transthoracic echocardiogram prior to intervention.
BMI: body mass index, eGFR: estimated glomerular filtration rate, MI: myocardial infarction, PCI: percutaneous coronary intervention, CABG: coronary artery bypass graft, EF: Ejection fraction, OT: occlusion time, LT: lysis time.
Normal values: haemoglobin 130–180 g/L in males and 115–165 g/L in females; haematocrit 40–52% in males and 36–47% in females; platelet count 150–400 × 10^9/L; creatinine 60–110 μmol/L in males and 45–90 μmol/L in females; C-reactive protein 0–5 mg/L.

are presented as mean and standard deviation when normally distributed, or as medians and interquartile range, when non-normally distributed. Where necessary, log transformations were employed. Dichotomous variables were compared using chi-square test. The analysis of variance (ANOVA) or Kruskal-Wallis test were used, dependent on distribution, to assess the differences in OT, OT drops and LT, between the three groups in response to different treatments. Analyses were performed with Stata version 15.1 (StataCorp, College Station, TX, USA), blinded to drug allocation.

3. Results

Baseline patient characteristics are shown in Table 1. The intra-assay CV was 6% for OT, 8% for LT and 11% for D, and the inter-assay CV was 7% for OT, 9% for LT and 13% for D. Baseline OT was normally distributed (Fig. 1A). There was no difference in baseline OT between groups prior to $P2Y_{12}$ medication or aspirin (Table 1). Compared to baseline, OT was significantly prolonged (exhibiting reduced platelet reactivity) by all $P2Y_{12}$ inhibitors (clopidogrel 378 ± 87 s vs. 491 ± 93 s, $p < 0.001$; ticagrelor 416 ± 122 s vs. 549 ± 121 s, $p < 0.001$; cangrelor 381 ± 146 s vs. 613 ± 210 s, $p < 0.001$) but not by aspirin (431 ± 104 s vs. 463 ± 99 s, $p = 0.07$) (Fig. 2). The magnitude of OT prolongation compared to baseline (ΔOT) was greater for cangrelor compared to clopidogrel and ticagrelor (clopidogrel 113 ± 117 s vs. ticagrelor 133 ± 125 s vs. cangrelor 232 ± 192 s, $p = 0.031$).

At baseline, the number of drops (D) between OT and complete occlusion was normally distributed in the whole group and in each of the three $P2Y_{12}$-inhibitor subgroups. There was no significant difference in D at baseline between the ~~three~~ groups (pre-aspirin 3.3 ± 1.6, pre-clopidogrel 4.3 ± 1.6, pre-ticagrelor 4.1 ± 2.4, pre-cangrelor 3.6 ± 2.0; $p = 0.722$). The change in D between baseline and on $P2Y_{12}$ inhibitors reflects the effect of the drug on thrombus stability. Whilst clopidogrel had no effect on D, ticagrelor and cangrelor both significantly increased D, reflecting increased instability of thrombus [D pre- and post-clopidogrel 4.3 ± 1.6 vs. 4.5 ± 1.4, $p = 0.833$; pre- and post-ticagrelor 4.1 ± 2.4 vs. 6.8 ± 5.1, $p = 0.048$; pre- and post-cangrelor 3.6 ± 2.0 vs. 7.9 ± 8.9, $p = 0.046$] (Fig. 3). The change in drops between pre- and post-$P2Y_{12}$ inhibition (ΔD) was significantly different among the groups with the magnitude of the effect being greater for cangrelor than ticagrelor [clopidogrel 0.2 ± 1.8 vs. ticagrelor 2.7 ± 4.7 vs. cangrelor 4.3 ± 7.1, $p = 0.001$].

There was no relationship between the extent of OT prolongation from baseline (ΔOT) and the change in drops (ΔD pre- and post-$P2Y_{12}$ inhibitor) ($r = 0.193$, $p = 0.104$). There was a moderate correlation between OT pre-$P2Y_{12}$ inhibitor and D pre- $P2Y_{12}$ inhibitor ($r = 0.477$, $p = 0.0004$) and between OT post-$P2Y_{12}$ inhibitor and D post- $P2Y_{12}$ inhibitor ($r = 0.424$, $p = 0.002$).

Baseline LT was non-normally distributed (Fig. 1B). There was no difference in baseline LT between groups prior to $P2Y_{12}$ inhibitors or aspirin (Table 1). Compared to baseline, LT was reduced (showing enhanced endogenous fibrinolysis) after $P2Y_{12}$ inhibitors (1659[1318–2057]s vs. 1505[1296–1975]s, $p = 0.029$) [Fig. 1B]. This was due to cangrelor, which was the only $P2Y_{12}$ inhibitor to reduce LT (LT pre- and post-clopidogrel 1826[1320–2279]s vs. 1910[1417–2206]s, $p = 0.941$; pre- and post-ticagrelor 1522[1347–1915]s vs. 1466[1207–1880]s, $p = 0.360$; pre- and post-cangrelor 1622[1240–2048]s vs. 1388[960–1634]s, $p = 0.005$; pre- and post-aspirin 1920[1730–2481]s vs. 2057[1671–2440]s, $p = 0.478$) (Figs. 4 and 5). The change in LT between pre- and post-$P2Y_{12}$ inhibition (ΔLT)

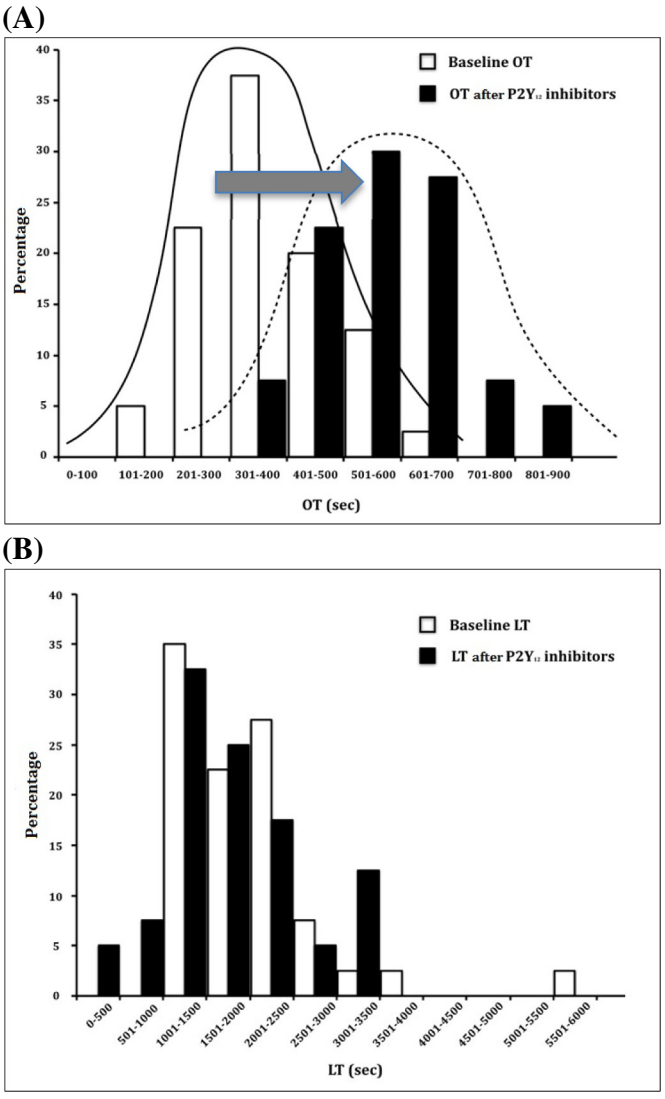

Fig. 1. Effect of $P2Y_{12}$ inhibitors on thrombotic status.
Distribution of occlusion time (OT) and lysis time (LT) before and after $P2Y_{12}$ inhibitors. (A) OT at baseline, prior to commencing $P2Y_{12}$ inhibitor, and after being established on $P2Y_{12}$ inhibitor therapy, and (B) LT at baseline, prior to commencing $P2Y_{12}$ inhibitor and after being established on $P2Y_{12}$ inhibitor.
The addition of $P2Y_{12}$ inhibitor to aspirin resulted in significant prolongation of OT (showing reduced platelet reactivity), as evidenced by a rightward shift (higher OT, representing reduced platelet reactivity). Lysis time was slightly reduced (showing enhanced endogenous fibrinolysis) after addition of $P2Y_{12}$ inhibitor to aspirin (lower LT, representing faster lysis).

was significantly different between the three groups (clopidogrel 30[−312–296]s vs. ticagrelor 71[−92–246]s vs. cangrelor 364[133–686]s, $p = 0.013$). All variables in Table 1 were interrogated for effects on baseline OT and LT using relevant parametric and non-parametric tests and no significant relationships were found.

4. Discussion

This is the first study demonstrating the effects of $P2Y_{12}$ inhibitors on thrombus stability under high shear conditions, in native blood. In this small pilot study, we show that we can assess thrombus stability using a simple technique and that $P2Y_{12}$ inhibitors inhibit shear-induced platelet reactivity with the magnitude of effect being greatest for cangrelor, followed by ticagrelor, and clopidogrel. Cangrelor and ticagrelor significantly de-stabilize thrombus formation at high shear and

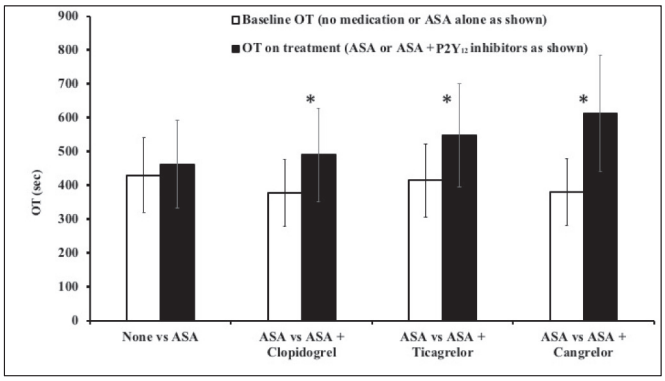

Fig. 2. Occlusion time at baseline and after $P2Y_{12}$ inhibitors and aspirin.
Occlusion time (OT) was significantly prolonged (reduced platelet reactivity) in patients on established dual antiplatelet therapy. The magnitude of OT prolongation was greater for cangrelor compared to clopidogrel and ticagrelor. Baseline OT is indicated by open bars; OT on treatment with aspirin (ASA) alone or $P2Y_{12}$ inhibitors + aspirin is indicated by solid bars. * $p < 0.001$.

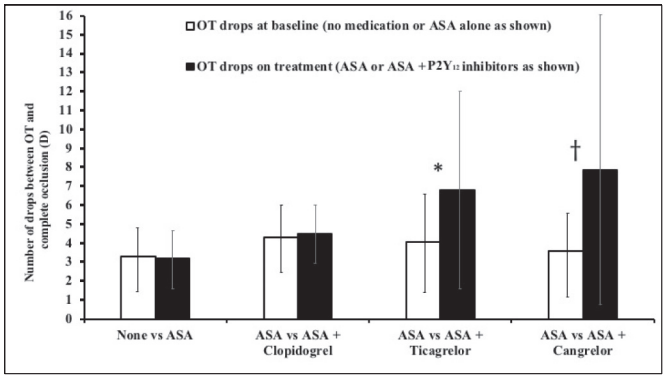

Fig. 3. Effect of $P2Y_{12}$ inhibitors on thrombus stability.
Number of drops (D) measured between recorded occlusion time (OT) and complete occlusion were significantly prolonged compared to baseline (reflecting greater thrombus instability) after treatment with ticagrelor and cangrelor. Mean number of drops for the OT at baseline is indicated by open bars and after $P2Y_{12}$ inhibitor is indicated by solid bars. * $p = 0.048$ † $p = 0.046$.

cangrelor also reduced endogenous lysis time.

Whilst the effect of $P2Y_{12}$ inhibitors on in vitro thrombus stability is related to the inhibition of platelet reactivity, demonstrated by the moderate correlation between occlusion time and the number of drops, the effect of $P2Y_{12}$ inhibitor on platelet reactivity does not always mirror the effect on in vitro thrombus stability. This is important, as it has always been assumed that inhibition of platelet aggregation is the best measure of the effectiveness of $P2Y_{12}$ inhibitor treatment. The lack of effect of aspirin alone on OT is in concordance with previous findings showing that at pathologically relevant high shear rates, aspirin has little or no effect on thrombus formation [9–11].

The relative magnitude of the impact of $P2Y_{12}$ inhibitors on thrombus stability is novel and important. Prior experimental data, predominantly in ex vivo perfusion models, suggest that some antithrombotic therapies may act by increasing the fragility of the growing thrombus or enhancing endogenous fibrinolytic activity. Clopidogrel has been shown to greatly enhance platelet-thrombus disaggregation in a perfusion chamber; and it has been proposed that measurement of disaggregation may be superior to measures of peak aggregation as a way of identifying clopidogrel responsiveness [12,13]. In response to ex vivo ADP stimulation, blood from patients taking clopidogrel formed loosely packed, unstable thrombi, compared to blood from untreated patients, indicating that clopidogrel impaired the formation of the platelet-to-platelet contacts needed for normal thrombus growth and

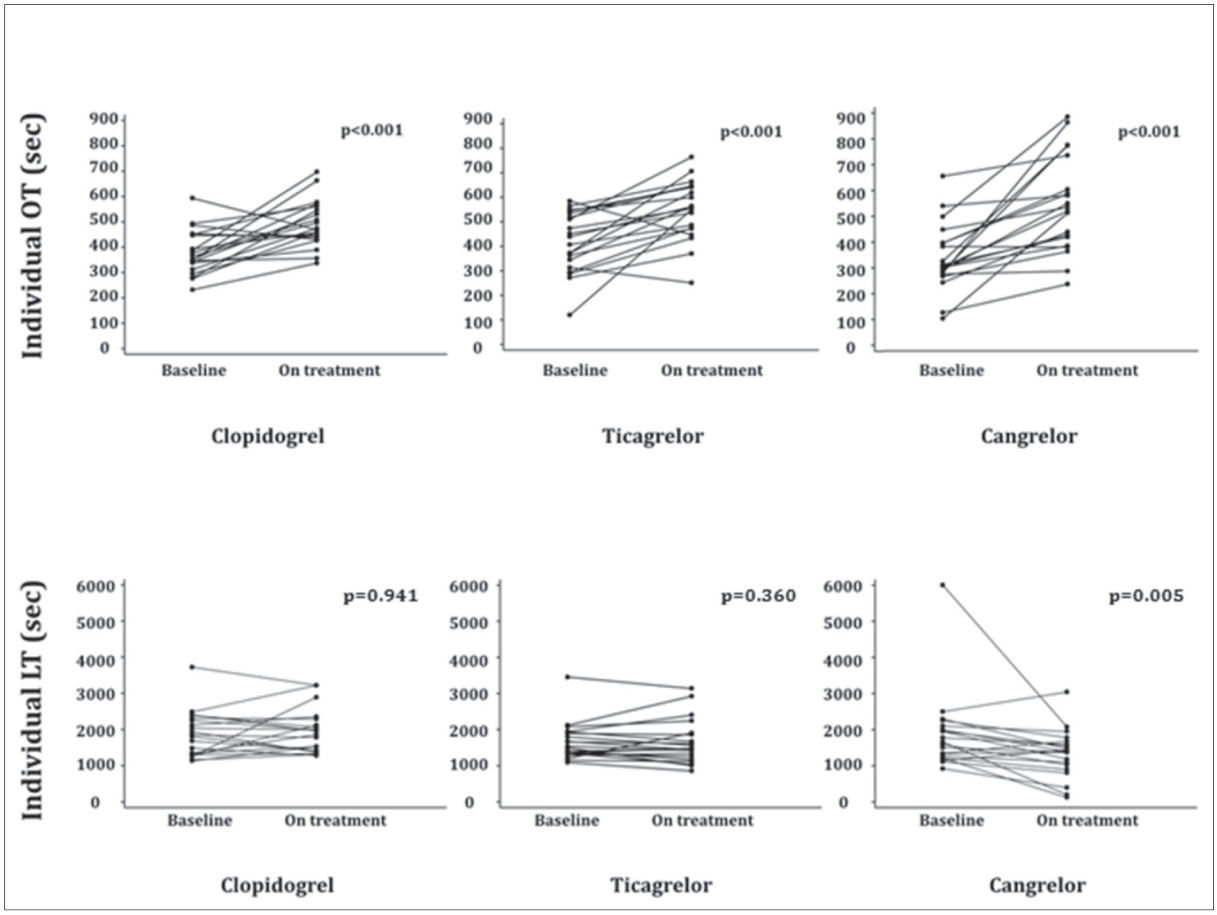

Fig. 4. Effect of $P2Y_{12}$ inhibitors on occlusion and fibrinolysis in vitro.
Occlusion time was significantly prolonged (exhibiting reduced platelet reactivity) by all $P2Y_{12}$ inhibitors. Cangrelor was the only $P2Y_{12}$ inhibitor to enhance fibrinolysis (as shown by shortened lysis time, LT).

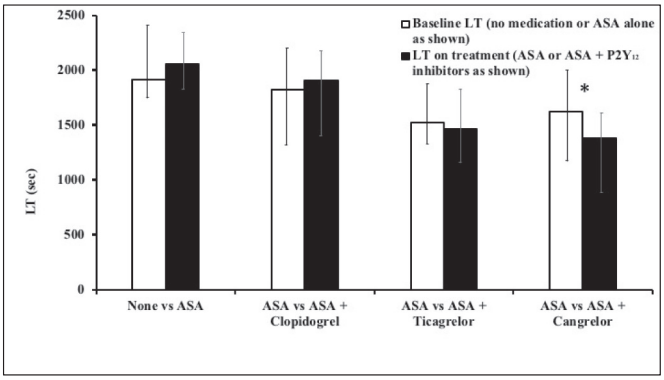

Fig. 5. Effect of $P2Y_{12}$ inhibitors and aspirin on endogenous fibrinolysis.
Compared to baseline, cangrelor was the only $P2Y_{12}$ inhibitor to significantly enhance endogenous fibrinolysis (as evidenced by reduced lysis time [LT]). Baseline LT is indicated by open bars and LT on treatment with aspirin (ASA) alone or aspirin + $P2Y_{12}$ inhibitors is indicated by solid bars. * $p = 0.005$.

stabilization [14]. Whilst $P2Y_{12}$ inhibitors variably reduced the rate of thrombus growth, they consistently reduced thrombus stability and induced thrombus fragmentation [15]. In an in vivo mesenteric artery injury model in $P2Y_{12}$-null mice, only small unstable thrombi formed, which did not reach occlusive size [16]. $P2Y_{12}$ inhibitors and phosphatidyl-inositol 3-kinase inhibitors have been shown to destabilize platelet-platelet contacts, leading to disaggregation and embolization [17–19]. A recent observational study indicates that the longer the time [20] between $P2Y_{12}$ inhibitor administration and PPCI, the better the pre-intervention epicardial flow in the infarct-related artery suggesting that $P2Y_{12}$ inhibitors may exert a thrombus de-stabilizing effect. In a murine model, cangrelor was strongly protective against ischaemic reperfusion injury [21], an effect that was dependent on the presence of blood, and likely platelets, via the $P2Y_{12}$ receptor. Clopidogrel has similar cardioprotective properties [22].

The mechanism through which $P2Y_{12}$ inhibitors destabilize thrombus is not fully understood. Adenosine diphosphate (ADP) plays an important role in the maintenance of thrombus stability [23] and the $P2Y_{12}$ receptor is required for irreversible platelet aggregation and thrombus stabilization [24]. Platelets also contain the vast majority (~90%) of plasminogen activator inhibitor (PAI)-1 present in blood. Thrombin can induce the release of PAI-1 together with thrombin activatable fibrinolysis inhibitor from activated platelets [25], therefore thrombin inhibitors may exert a fibrinolytic effect. Of the $P2Y_{12}$ inhibitors studied here, only cangrelor enhanced endogenous fibrinolysis, although a trend was observed with ticagrelor which may have been significant had the sample size increased, or compliance been mandated, especially since both ticagrelor and cangrelor de-stabilised thrombus formation.

$P2Y_{12}$ inhibitors differ in their speed of onset and magnitude effect. Clopidogrel achieves variable platelet inhibition and maximum inhibition of platelet aggregation (IPA) within 3 to 7 days [26–29]. Ticagrelor achieves maximum IPA after 2 h [30–33], whilst cangrelor achieves extensive IPA within 2 min of bolus administration and maximal plasma concentration within 10 min [34]. At steady state, IPA is higher with ticagrelor than clopidogrel (~90–95% vs. ~60%) [31,32].

However, cangrelor is more potent than ticagrelor [35] and produces profound IPA (96.0 ± 8.6%) [34,36,37].

The main limitation of this study is the small sample size, but was justified by the adoption of within-group design, as patients acted as self-controls. However, studies with small sample size generally have high variability and sampling bias and may not be representative of the general population. Second, for orally-administered $P2Y_{12}$ inhibitors, the drug plasma concentrations were not measured to assess compliance, which was only ascertained by asking the patient. This is particularly relevant to clopidogrel which exerts highly variable pharmacodynamic response with some 20–30% of patients showing limited inhibition of ADP-induced platelet aggregation [38,39]. Possible lack of compliance with aspirin and oral $P2Y_{12}$ inhibitors may have led to an overestimation of the relative magnitude of effect of cangrelor. Third, patients were only sampled once before and once on treatment, thus variability may have been caused by single time point sampling and within patient variation. Fourth, from the observed effects of $P2Y_{12}$ inhibitors on thrombus stability and fibrinolysis in stable patients, we cannot directly infer the effects of these agents in acute thrombotic states such as ACS. Furthermore, there may be interindividual variability in the observed effects of $P2Y_{12}$ inhibitors on fibrinolysis that may resemble other variable $P2Y_{12}$ inhibitor effects such as the genetic variability observed with clopidogrel absorption. Fifth, although patients were similar in the four groups with stable angina and allocated to different drug regimens by the clinical care teams and not related to this study, there is nevertheless a potential for bias by the clinical care team in allocation of $P2Y_{12}$ inhibitor. Sixth, the group tested before and on aspirin had lower incidence of prior coronary disease than the other 3 groups, although this group had a higher BMI and incidence of diabetes, so this could have confounded the results. Finally, all patients taking $P2Y_{12}$ inhibitors were also on aspirin and it is possible that the effects seen reflected not just $P2Y_{12}$ inhibitor effect but synergistic effect between aspirin and $P2Y_{12}$ inhibitor. However, since all patients on taking $P2Y_{12}$ inhibitors were also on aspirin, the additional effect of aspirin is likely a fixed variable so that the magnitude of effect of different $P2Y_{12}$ inhibitors can be compared. Finally, we did not compare the results of the GTT with routine coagulation tests or platelet aggregation tests since neither routine coagulation tests such as the activated partial thromboplastin time or prothrombin time [40], nor tests of platelet aggregation such as the results of VerifyNow and thromboelastography correlate with the results of the GTT [41,42]. The lack of correlation is likely attributable to a combination of the high shear used in the GTT (in comparison to much lower shear in VerifyNow and also thromboelastography) and that GTT employs native, non-anticoagulated blood, in contrast to the citrated blood used by other tests.

Nevertheless, this study demonstrates a relatively novel beneficial effect of $P2Y_{12}$ inhibitors on thrombus stability, with a significant effect of cangrelor on destabilising thrombus and enhancing endogenous fibrinolysis. Cangrelor use, partly due to cost, is predominantly restricted to STEMI situations where oral $P2Y_{12}$ inhibitor treatment is either not possible or absorption unreliable. Future studies are required to assess whether, if given early after symptom onset, cangrelor can exert favourable effects on myocardial salvage, through novel effects potentiating thrombus instability and susceptibility to fibrinolysis.

5. Conclusions

We demonstrate the ability to measure thrombus stability in vitro with a point-of-care technique and demonstrate that $P2Y_{12}$ inhibitors potentiate thrombus instability at high shear. Cangrelor, and to a lesser extent ticagrelor, significantly de-stabilises thrombus formation and cangrelor also enhanced endogenous fibrinolysis. The potentiation of thrombus instability could become a new pharmacological target, which may be particularly important in the setting of acute coronary syndromes.

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Conflict of interest

None.

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