# Thrombin formation and platelet activation at the site of vascular injury in patients with coronary artery disease treated with clopidogrel combined with aspirin

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**Abstract**

**Background:** Data on the effects of oral antiplatelet agents on blood coagulation in vivo are conflicting. The platelet glycoprotein (GP) IIIa PlA2 allele has been suggested to modulate antithrombotic actions of clopidogrel.

**Aim:** We investigated whether clopidogrel combined with aspirin affects local thrombin formation and platelet activation triggered by vascular injury.

**Method:** We studied patients with stable coronary artery disease on chronic aspirin therapy randomised to addition of clopidogrel 75 mg/d (n = 30) or continuation of aspirin 100 mg/d (n = 30) for 4 weeks. Markers of thrombin generation [thrombin-antithrombin complexes (TAT) and prothrombin 1.2 fragments (F1.2)] and markers of platelet activation [soluble CD40 ligand (sCD40L) and P-selectin] were determined in the supernatant of blood samples obtained from a microvascular injury site.

**Results:** Total amounts of thrombin markers produced at the site of injury were similar before and after addition of clopidogrel, whereas platelet release of sCD40L and P-selectin was lower during treatment with aspirin + clopidogrel by 33.8% and 27.8% ($p < 0.001$), respectively. Patients in the highest tertile of reduction in platelet activation had previous myocardial infarction and peripheral arterial disease and released the highest amounts of sCD40L and P-selectin at baseline. TAT and F1.2 generation as well as sCD40L or P-selectin release were not influenced by the presence of the PlA2 allele.

**Conclusion:** Our study shows that clopidogrel combined with aspirin does not reduce thrombin formation following vascular injury, but attenuates platelet sCD40L and P-selectin release.

**Key words:** clopidogrel, thrombin, coronary artery disease, aspirin, vascular injury, platelets

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## Introduction

Apart from inhibition of thromboxane $A_2$-induced platelet aggregation, aspirin at therapeutic doses has been shown to impair thrombin formation in healthy individuals and patients at an increased risk of cardiovascular disease [1]. The mechanism underlying reduction in thrombin generated during aspirin treatment involves the inhibition of platelet activation as activated platelets provide the catalytic surface to assemble the prothrombinase complex forming thrombin [2].

Clopidogrel, a thienopyridine derivative, inhibits adenosine diphosphate (ADP)-induced platelet aggregation through a specific action on the platelet $P2Y_{12}$ purinergic receptors. Addition of clopidogrel to aspirin has been shown to be associated with a further decline in the risk of cardiovascular complications, largely thromboembolic by nature, such as myocardial infarction (MI), stroke, or death in patients treated for acute coronary syndromes (ACS) and those undergoing percutaneous coronary interventions (PCI) [3].

One might suspect that, like aspirin [1], clopidogrel can attenuate thrombin formation. However, it has been reported that clopidogrel does not suppress circulating blood coagulation markers in aspirin-treated patients with non-ST-elevation ACS [4], patients with atrial fibrillation [5], or after peripheral endovascular interventions [6]. It is also unclear whether clopidogrel can modify blood release of platelet secretion products. In patients with stable

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coronary artery disease (CAD), clopidogrel reduced soluble CD40 ligand (sCD40L) levels [7, 8], but not after elective PCI [9]. Clopidogrel has been reported to attenuate CD40L and P-selectin expression on platelets and sCD40L levels in patients with ACS [10, 11].

Efficient haemostasis occurs only at vascular lesions where tissue factor (TF) is exposed and platelets rapidly aggregate. Therefore, measurements of thrombin or platelet markers in venous blood do not reflect coagulant reactions occurring in response to injury. A number of studies have shown that a model of microvascular injury enables a reliable qualitative and quantitative evaluation of thrombin generation and platelet activation as a function of time [12-17]. This model consists of performing standardised skin incisions by means of a bleeding-time device. Blood is collected at equal time intervals into cannulae, then passed into an anticoagulant solution, and in the separated supernatant several substances can be detected. Using this model, Welterman et al. [9] demonstrated no effect of 300 mg loading dose of clopidogrel on thrombin formation and platelet activation in patients undergoing elective coronary stenting. In contrast, clopidogrel given for 2 weeks has been reported to decrease thrombin generation triggered by vascular injury, but only in carriers of the platelet glycoprotein (GP) IIIa PlA2 allele [15].

The aim of the current study was to evaluate the effect of clopidogrel added to aspirin on thrombin generation and platelet activation at the site of haemostatic plug formation in stable CAD patients. We also sought to investigate whether indeed the presence of GPIIIa PlA2 allele affects thrombin formation in clopidogrel-treated subjects.

## Methods

### Patients

Sixty white male patients with documented CAD, i.e., with a history of MI (n = 42) and hospitalisation for unstable angina (n = 18), were enrolled in the study. Exclusion criteria were: diabetes mellitus, any acute illness, cancer, acute coronary syndrome or PCI within the previous 6 months, hepatic or renal dysfunction, anticoagulant therapy, previous coronary bypass graft surgery, a history of venous thromboembolism. All were treated with aspirin 75 mg/d for ≥ 6 months.

Patients were randomised in a 1 : 1 ratio using computerised random number generation by an independent investigator on an open-label basis to addition of clopidogrel (Plavix, Sanofi-Aventis) 75 mg once daily or continuation of aspirin (Aspirin Protect, Bayer, Germany) 100 mg once daily for 4 weeks. Compliance with medication usage was assessed from the number of tablets returned at each visit in both groups. The last tablets were taken in the presence of an investigator 2-4 h before blood sampling.

All subjects enrolled in this study provided written informed consent. The University Ethical Committee approved the study.

### Laboratory investigations

Fasting blood samples were obtained twice in each patient (before and after intervention) from an antecubital vein using a 21-gauge butterfly needle. Lipid profile, C-reactive protein, glucose, creatinine, and platelet count were determined using routine laboratory methods.

Blood samples for thrombin and platelet markers were centrifuged at 2500 g for 15 min and plasma was stored at –80°C for subsequent batch analysis. In plasma obtained from peripheral venous blood, we determined using commercially available ELISAs: thrombin-antithrombin complexes (TAT) and prothrombin 1.2 fragment (F1.2), markers of thrombin formation (Enzygnost, Dade Behring, Marburg, Germany), as well as sCD40L and P-selectin, markers of platelet activation (R & D Systems, Abingdon, UK). For sCD40L measurements, blood was drawn into tubes with EDTA as an anticoagulant, and then tubes were put immediately on ice. In the remaining 3 measurements we used citrated plasma (0.105 M sodium citrate, 1 vol./ /9 vol. of blood). Serum thromboxane $B_2$, an indicator of aspirin's action in vivo, was measured after 60 min of blood clotting at 37°C using an ELISA kit (Cayman Chemicals) in all 60 patients, yielding values ranging from 0 to 0.89 ng/ml.

Inter-assay and intra-assay variability for the ELISA results obtained in venous blood samples were from 5.1 to 6.5%.

### Model of vascular injury

To assess local haemostatic response to vascular injury, we analysed the blood samples collected at 60-second intervals from the standardised skin incision on the lateral aspect of a forearm, made using a Simplate IR device (Organon Teknika, Durham, NC) at the inflation of the sphygmomanometer cuff at 40 mmHg as previously described in detail [13, 16, 17]. Blood was collected from the edge of the wound by means of heparinised tubes of known diameter (Kabe Labortechnik, Numbrecht-Elsenroth, Germany). After measuring the height of the blood column, the blood was passed into Eppendorf tubes containing anticoagulants according to the procedure described previously [16, 17]. All procedures were performed by the same investigator blinded to the status of the patient studied. After centrifugation at 3000 g at 4°C for 20 min, the supernatant was aliquoted and frozen at –80°C. The tests were performed on freshly thawed samples. All 4 haemostatic markers, i.e., TAT and F1.2 (Dade Behring), plus sCD40L and P-selectin (R & D Systems) were measured in the supernatant samples of the bleeding-time blood by blinded technicians. Inter-assay and intra-assay variability for values measured in the bleeding-time blood samples were from 5 to 7%.

To assess the kinetics of thrombin formation and platelet activation at the site of injury, we used two variables, i.e., maximum velocity and total amounts of each marker produced within 6 min of bleeding as described previously [13, 16, 17]. Experimental values were fitted to second-order polynomials using a SigmaPlot computer program. Since the curve fitting of the data provided excellent fits for the experimental data, we calculated concentrations of each marker every 15 seconds. The maximum rate of the increase in concentrations of each marker was estimated by analysis of the rates in all 15-second intervals. The area under the curve of a total amount (concentration multiplied by blood volume of each sample) versus time was regarded as the measure of overall local mediator production [16]. Analyses were limited to the first 6 min because minimum bleeding time was 6 min in all subjects.

### Genetic analysis

The $\beta_3$ integrin PlA1A2 polymorphism was determined as previously described [17].

### Statistical analysis

The Kolmogorov-Smirnov test was used to assess conformity with a normal distribution. The Mann-Whitney U test or Student's t test was used to test differences between groups as appropriate. Data obtained before and after intervention were compared by the Wilcoxon matched pairs test or Student's paired t test as appropriate. No correction was made for multiple testing. The $\chi^2$ test or Fisher's exact test was used to compare categorical variables. All tests were two-sided. A p value < 0.05 was considered significant.

The study was powered to have an 80% chance of detecting a 10% reduction in total TAT generation at the site of microvascular injury following one-month therapy in a randomised setting using a p value of 0.05, based on mean values of aspirin-induced effects on thrombin generation in published papers [16, 17]. In order to demonstrate such a change or greater, 27 patients were required in each group. The corresponding number of patients for total sCD40L release in this coagulation model was calculated to be 29.

## Results

There were no significant differences in baseline characteristics between patients randomised to aspirin alone and those randomised to aspirin + clopidogrel (Table I). Both thrombin and platelet markers determined in venous blood were similar, regardless of clopidogrel administration or not (Table I).

### Markers of thrombin generation

At the site of microvascular injury, clopidogrel added to aspirin was associated with decreases in TAT (Figure 1 A) and F1.2 (Figure 1 C) concentrations in bleeding-time blood. Maximum rates of TAT formation expressed as a concentration versus time fell following clopidogrel from 0.124 ± 0.015 to 0.083 ± 0.009 nmol/l/s (p = 0.01), while corresponding values for F1.2 were 0.371 ± 0.045 and 0.236 ± 0.039 nmol/l/s (p = 0.002). A peak rate of TAT and F1.2 formation expressed as total amounts versus time did not differ before and after addition of clopidogrel (4.65 ± 0.45 vs. 4.43 ± 0.49 fmol/s for TAT, p = 0.6, and 7.18 ± 0.72 vs. 8.23 ± 0.79 fmol/s for F1.2, p = 0.4). Total amounts of TAT and F1.2 generated at the site of injury within 6 min remained unaltered after addition of clopidogrel to aspirin (2893 ± 372 vs. 2912 ± 329 fmol, p = 0.8, and 6823 ± 892 vs. 6342 ± 737 fmol; p = 0.6, respectively). No changes in TAT and F1.2 generation were found after an additional 4 weeks of aspirin administration (Figure 2 A and C).

### Markers of platelet activation

Platelet protein secretion was suppressed following addition of clopidogrel to aspirin (Figure 1 B and D). Maximum rates of sCD40L and P-selectin release expressed as total amounts versus time were reduced following clopidogrel (1.14 ± 0.12 vs. 0.72 ± 0.09 pg/s; p < 0.001, and 0.19 ± 0.02 vs. 0.12 ± 0.01 pg/s; p < 0.001, respectively) as well as the velocity of increase in sCD40L and P-selectin concentrations (0.056 ± 0.009 vs. 0.021 ± 0.005 ng/ml/s, p < 0.001, and 5.29 ± 0.69 vs. 2.62 ± 0.34 ng/ml/s, p < 0.001, respectively). Total amounts of both platelet markers collected at the site of injury within the first 6 min of bleeding were markedly reduced, by 33.8% to 602.3 ± 68.1 pg for sCD40L (p < 0.001) and by 27.8% to 93.4 ± 10.2 ng for P-selectin (p = 0.005) following addition of clopidogrel. Aspirin taken for an additional 4 weeks did not affect platelet release of sCD40L (Figure 2 B) or P-selectin in our model (Figure 2 D).

Patients in the highest tertile of the magnitude of clopidogrel-associated reduction in sCD40L release within 6 min of bleeding (by 38 vs. 19% for two remaining tertiles; p < 0.001) had previous MI and PAD. These individuals also had the highest baseline amounts of both platelet markers measured within the first 6 min of bleeding (989.4 ± 97.1 vs. 832.6 ± 76.4 pg for sCD40L, p = 0.012, and 145.1 ± 13.6 vs. 113.9 ± 10.5 ng for P-selectin, p = 0.024). After clopidogrel addition platelet protein secretion was similar in all the tertiles. They did not differ from the remaining 20 patients in terms of demographics, lipid profile, medications, and plasma levels of all 4 markers studied (data not shown).

### Effect of the PlA2 allele

There were 10 patients heterozygous for the PlA1A2 polymorphism (no PlA2A2 homozygotes) both among patients receiving clopidogrel + aspirin and those on aspirin alone. Carriers of the PlA2 allele and PlA1A1 homozygotes

**Table I.** Baseline characteristics

| | Aspirin + clopidogrel (n = 30) | Aspirin (n = 30) | p |
|---|---|---|---|
| Age [years] | 57.4 ± 12.3 | 57.8 ± 14.1 | 0.93 |
| BMI [kg/m$^2$] | 27.2 ± 3.1 | 26.9 ± 3.3 | 0.87 |
| Current smokers, n (%) | 10 (33) | 11 (36.7) | 0.91 |
| Hypertension, n (%) | 18 (53.3) | 21 (70) | 0.72 |
| PAD, n (%) | 10 (33.3) | 10 (33.3) | 1 |
| Previous MI, n (%) | 14 (46.7) | 16 (53.3) | 0.84 |
| Previous PCI, n (%) | 18 (53.3) | 24 (80) | 0.39 |
| Medications | | | |
| aspirin, n (%) | 30 (100) | 30 (100) | 1 |
| statins, n (%) | 24 (80) | 21 (70) | 0.82 |
| beta-blockers, n (%) | 20 (66.7) | 23 (76.7) | 0.77 |
| ACEI, n (%) | 13 (43.3) | 14 (46.7) | 0.93 |
| Laboratory parameters | | | |
| TC [mmol/l] | 5.23 ± 1.09 | 5.28 ± 0.98 | 0.72 |
| LDL-C [mmol/l] | 3.17 ± 0.83 | 3.21 ± 0.79 | 0.81 |
| HDL-C [mmol/l] | 1.44 ± 0.38 | 1.41 ± 0.33 | 0.72 |
| TG [mmol/l] | 1.36 ± 0.54 | 1.45 ± 0.53 | 0.46 |
| platelets [10$^9$/l] | 239 (202-287) | 234 (199-276) | 0.79 |
| CRP [mg/l] | 2.1 (1.3-3.2) | 2.4 (0.9-3.4) | 0.58 |
| TAT [μg/l] | 3.23 (2.47-4.18) | 3.34 (2.41-4.04) | 0.47 |
| F1.2 [nmol/l] | 0.82 (0.61-0.97) | 0.78 (0.64-1.01) | 0.61 |
| sCD40L [ng/ml] | 0.42 (0.33-0.59) | 0.39 (0.32-0.56) | 0.84 |
| P-selectin [ng/ml] | 69.2 (49.2-87.9) | 71.5 (50.6-83.3) | 0.77 |
| Bleeding time [s] | 431.6 ± 87.5 | 436.2 ± 63.3 | 0.85 |
| $TXB_2$ [ng/ml] | 0.54 (0.21-0.79) | 0.65 (0.28-0.75) | 0.68 |

*Abbreviations: BMI – body mass index, PAD – peripheral arterial disease (ankle brachial index < 0.9), MI – myocardial infarction, PCI – percutaneous coronary intervention (11 to 27 months before the study), ACEI – angiotensin-converting enzyme inhibitors, TC – total cholesterol, LDL-C – low-density lipoprotein cholesterol, HDL-C – high-density lipoprotein cholesterol, TG – triglycerides, CRP – C-reactive protein, TAT – thrombin-antithrombin complexes in venous blood, F1.2 – prothrombin 1.2 fragments in venous blood, sCD40L – soluble CD40 ligand in venous blood, $TXB_2$ – thromboxane $B_2$*
*Values are given as mean ± SD, median (interquartile range), or number (percentage)*

did not differ with regard to demographic, clinical and laboratory variables (data not shown). None of the variables describing our coagulation model for TAT and F1.2 generation as well as sCD40L or P-selectin release were influenced by the presence of the PlA2 allele (data not shown).

## Discussion

This study shows that in vivo, at the site of injury, clopidogrel does not affect thrombin formation in CAD patients receiving aspirin, regardless of the presence or absence of the PlA2 allele, but significantly reduces platelet activation as evidenced by decreased local release of sCD40L and P-selectin. These observations expand our knowledge based on previous small studies in patients taking clopidogrel alone [15] or those undergoing PCI on dual therapy [9].

Our findings are in agreement with the study by Weltermann et al. [9] using a similar model. Using a concentration-based analysis of the bleeding-time blood samples, Dropinski et al. [15] reported clopidogrel-related reduction in thrombin generation in CAD patients with normal lipid profile, who did not take aspirin and were carriers of the PlA2 allele. However, concentrations of any analyte in the bleeding-time blood samples, when their volumes are significantly increased as in clopidogrel-treated subjects, do not provide reliable information on thrombin generation. Therefore, we analysed the total amounts of thrombin generated following vascular injury to avoid falsely reduced values of the markers measured in shed blood. Moreover, our real-life CAD patients had multiple risk factors and received drugs recommended in the cardiovascular prevention guidelines, including aspirin.

One might speculate that because aspirin itself may decrease thrombin generation triggered by vascular injury [1], a further reduction cannot be achieved upon addition of clopidogrel. To test this hypothesis in patients with established CAD, unethical discontinuation of aspirin would be necessary.

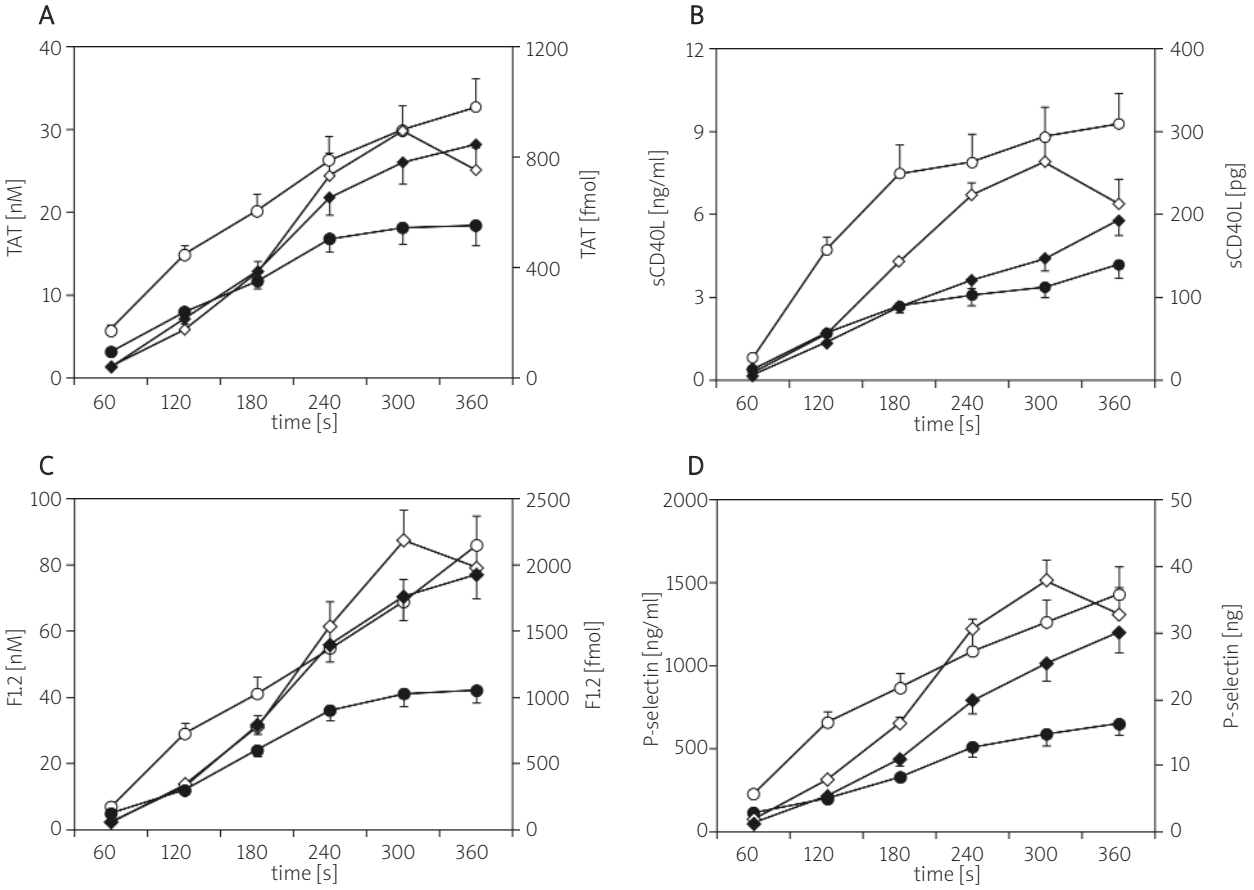


**Figure 1.** Thrombin formation and platelet protein secretion at the site of microvascular injury in patients with stable coronary artery disease: effect of addition of clopidogrel to aspirin (75 mg/d). Concentrations (circles) and total amounts (diamonds) of thrombin-antithrombin (TAT) complexes (**A**), soluble CD40 ligand (sCD40L) (**B**), prothrombin fragment 1.2 (F1.2) (**C**), and P-selectin (**D**) in the 60-second bleeding-time blood samples in 30 patients before (open symbols) and after addition of clopidogrel (75 mg/d) to aspirin (closed symbols). Values are plotted as means ± SEM

As an antagonist of platelet ADP receptors, clopidogrel has the potential to reduce platelet expression of CD40L and P-selectin [10, 11]. Since aspirin does not reduce CD40L or P-selectin expression [10], the reduction in the release of both markers at the site of injury most likely results from clopidogrel administration and explains at least in part the additional benefits from the dual antiplatelet therapy, also indirectly by suppressed platelet release of inflammatory mediators such as sCD40L [18]. We first demonstrated that clopidogrel added to aspirin can reduce amounts of P-selectin released at the site of vascular injury, as suggested by previous studies [11], but not all [4]. Reductions in sCD40L and P-selectin release associated with clopidogrel administration showed a relatively small variability in stable CAD patients using our approach. Low variability in platelet activation in CAD was also demonstrated in a study by Serebruany et al. [19], although they did not measure P-selectin or sCD40L levels. Our findings differed from observations in 20 patients after elective PCI within the first 48 hours in whom platelet β-thromboglobulin in shed blood was unaltered following clopidogrel [9]. A different study design presented here (longer study duration, other platelet markers, a different way of data analysis) may explain this discrepancy.

Clopidogrel-treated patients in our study had more than two times longer bleeding time, a measure of overall platelet haemostatic function, compared to those only on aspirin as shown previously [20]. This confirmed a strong antiplatelet action of clopidogrel in vivo.

Interestingly, clopidogrel added to aspirin resulted in greater reduction in platelet protein secretion upon injury in MI survivors with PAD who had the highest baseline platelet activation. In contrast to ADP-induced platelet aggregation [21], a change in release of sCD40L and P-selectin following clopidogrel addition was associated with baseline platelet activation and the extent of atherosclerotic vascular disease. Contrary to patients evaluated 24 h after coronary stenting [22], in stable CAD

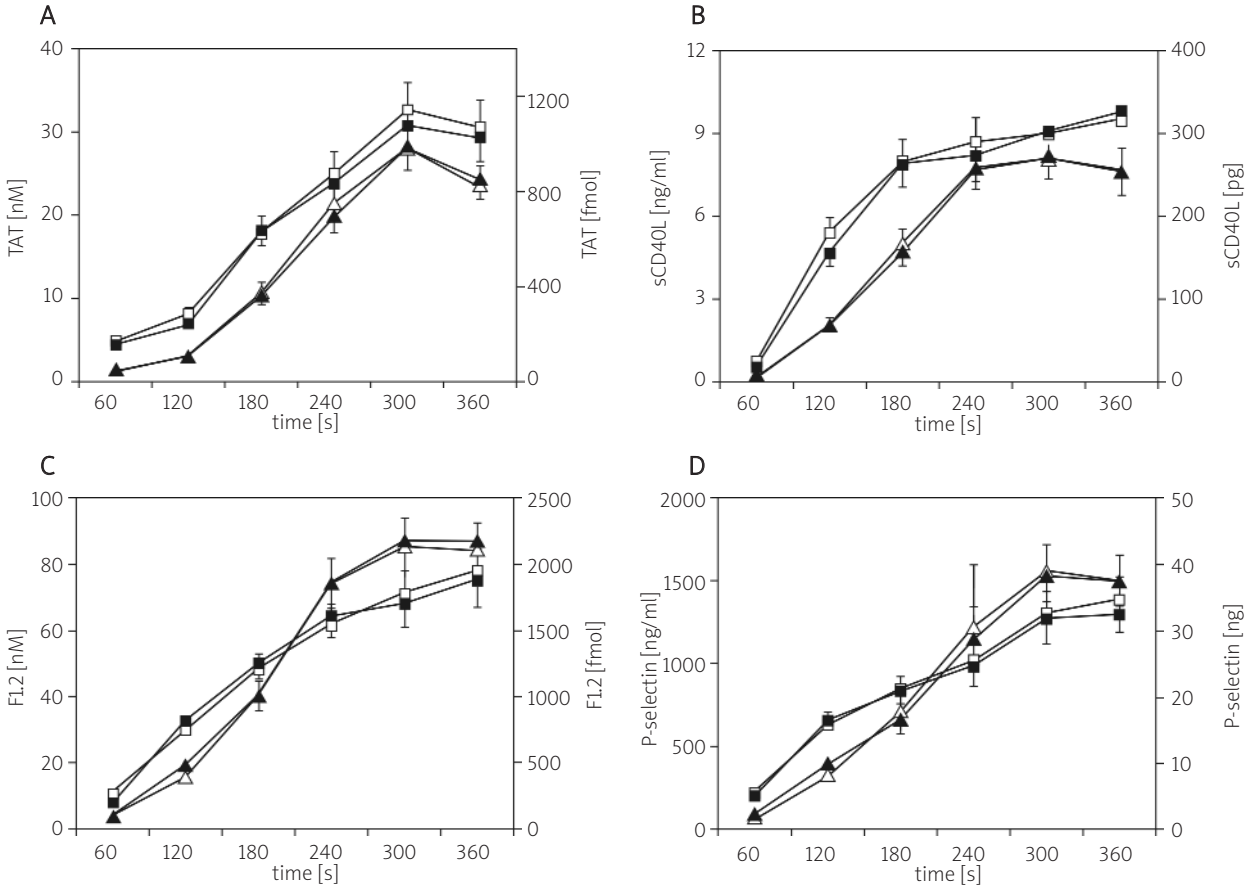


**Figure 2.** Thrombin formation and platelet protein secretion at the site of microvascular injury in patients with stable coronary artery disease: effect of chronic aspirin 75 vs. 100 mg/d treatment. Concentrations (squares) and total amounts (triangles) of thrombin-antithrombin (TAT) complexes (**A**), soluble CD40 ligand (sCD40L) (**B**), prothrombin fragment 1.2 (F1.2) (sCD40L) (**C**), and P-selectin (**D**) in 30 patients before (open symbols) and after treatment with aspirin 100 mg/d alone for an additional 4 weeks (closed symbols). Values are plotted as means ± SEM

patients on aspirin, clopidogrel abolished pre-treatment differences in platelet activation, leading to similar sCD40L and P-selectin values on dual therapy.

Another issue is whether poor responsiveness to antiplatelet agents might explain the lack of a significant reduction in thrombin formation on dual therapy. Low thromboxane levels in all patients indicate normal response to aspirin. Poor response to clopidogrel in a vast majority of CAD patients appears unlikely given the significant decreases in amounts of sCD40L and P-selectin released during aspirin + clopidogrel administration. Since some variability in clopidogrel-induced changes in platelet protein release has been observed, further studies regarding clopidogrel resistance in our model are warranted.

No effect of the PlA2 allele on platelet activation in our study corroborates the concept that response to antiplatelet drugs is determined by a number of factors and cannot be linked to single gene mutations [23].

Some study limitations should be acknowledged. The number of study participants was limited. However, our study was adequately powered to detect clopidogrel-associated reduction in platelet activation in shed blood, which suggests that a similar effect on thrombin cannot be overlooked. Second, the effects of clopidogrel and aspirin cannot be separated, as patients took both drugs. Third, direct platelet markers such as P-selectin expression detected using flow cytometry have not been analysed. Finally, analysis of whole blood clotting using thromboelastography, which was shown to be altered following clopidogrel given in PCI patients, suggesting impaired post-treatment thrombin generation [24], has not been performed. Volumes of bleeding-time blood are too small to perform thrombelastographic measurements.

We conclude that patients with CAD receiving both clopidogrel and aspirin produced similar amounts of thrombin following vascular injury, compared to subjects taking aspirin alone, without effects of the PlA1A2

polymorphism. However, dual antiplatelet therapy suppressed platelet protein release at the site of injury. This indicates that beneficial clinical outcomes of clopidogrel in combination with aspirin in CAD patients are largely associated with its strictly antiplatelet properties.

## References


1. Undas A, Brummel-Ziedins KE, Mann KG. Antithrombotic properties of aspirin and resistance to aspirin: beyond strictly antiplatelet actions. *Blood* 2007; 109: 2285-92.
2. Monroe DM, Hoffman M, Roberts HR. Platelets and thrombin generation. *Arterioscler Thromb Vasc Biol* 2002; 22: 1381-9.
3. Eshaghion S, Kaul S, Amin S, et al. Role of clopidogrel in managing atherothrombotic cardiovascular disease. *Ann Intern Med* 2007; 146: 434-41.
4. Eikelboom JW, Weitz JI, Budaj A, et al. for the Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) Study Investigators. Clopidogrel does not suppress blood markers of coagulation activation in aspirin-treated patients with non-ST-elevation acute coronary syndromes. *Eur Heart J* 2002; 23: 1771-9.
5. Müller I, Massberg S, Zierhut W, et al. Effects of aspirin and clopidogrel versus oral anticoagulation on platelet function and on coagulation in patients with nonvalvular atrial fibrillation (CLAFIB). *Pathophysiol Haemost Thromb* 2002; 32: 16-24.
6. Cassar K, Bachoo P, Ford I, et al. Clopidogrel has no effect on D-dimer and thrombin-antithrombin III levels in patients with peripheral arterial disease undergoing peripheral percutaneous transluminal angioplasty. *J Vasc Surg* 2005; 42: 252-8.
7. Azar RR, Kassab R, Zoghbi A, et al. Effects of clopidogrel on soluble CD40 ligand and on high-sensitivity C-reactive protein in patients with stable coronary artery disease. *Am Heart J* 2006; 151: 521.e1-4.
8. Stellbaum C, Willich T, Boltzen U, et al. Clopidogrel-mediated reduction of circulating tissue factor in patients with stable coronary artery disease. *Eur J Haematol* 2007; 78: 347-52.
9. Welterman A, Fritsch P, Kyrle PA, et al. Effects of pretreatment with clopidogrel on platelet and coagulation activation in patients undergoing elective coronary stenting. *Thromb Res* 2003; 112: 19-24.
10. Quinn MJ, Bhatt DL, Zidar F, et al. Effect of clopidogrel pretreatment on inflammatory marker expression in patients undergoing percutaneous coronary intervention. *Am J Cardiol* 2004; 93: 679-84.
11. Xiao Z, Theroux P. Clopidogrel inhibits platelet-leukocyte interactions and thrombin receptor agonist peptide-induced platelet activation in patients with an acute coronary syndrome. *J Am Coll Cardiol* 2004; 43: 1982-8.
12. Weiss HJ, Lages B. Evidence for tissue factor-dependent activation of the classic extrinsic coagulation mechanism in blood obtained from bleeding time wounds. *Blood* 1988; 71: 629-35.
13. Undas A, Brummel KE, Musial J, et al. Simvastatin depresses blood clotting by inhibiting activation of prothrombin, factor V, and factor XIII and by enhancing factor Va inactivation. *Circulation* 2001; 103: 2248-53.
14. Undas A, Stępień E, Nizankowski R, et al. Effects of simvastatin on angiogenic growth factors released at the site of microvascular injury. *Thromb Haemost* 2006; 96: 342-7.
15. Dropinski J, Musial J, Jakiela B, et al. Anti-thrombotic action of clopidogrel and PI (A1/A2) polymorphism of beta3 integrin in patients with coronary artery disease not being treated with aspirin. *Thromb Haemost* 2005; 94: 1300-5.
16. Undas A, Brummel K, Musial J, et al. Blood coagulation at the site of microvascular injury: effects of low-dose aspirin. *Blood* 2001; 98: 2423-31.
17. Undas A, Brummel K, Musial J, et al. PlA2 polymorphism of b3 integrins is associated with enhanced thrombin generation and impaired antithrombotic action of aspirin at the site of microvascular injury. *Circulation* 2001; 104: 2666-72.
18. Aukrust P, Damas JK, Solum NO. Soluble CD40L and platelets: self-perpetuating pathogenic loop in thrombosis and inflammation? *J Am Coll Cardiol* 2004; 43: 2326-8.
19. Serebruany VL, Malinin AI, Atar D, et al. Consistent platelet inhibition during long-term maintenance-dose clopidogrel therapy among 359 compliant outpatients with documented vascular disease. *Am Heart J* 2007; 153: 371-7.
20. Payne DA, Hayes PD, Jones CI, et al. Combined therapy with clopidogrel and aspirin significantly increases the bleeding time through a synergistic antiplatelet action. *J Vasc Surg* 2002; 35: 1204-9.
21. Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005; 45: 246-51.
22. Gurbel PA, Blinden KP, Hiatt BL, et al. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003; 107: 2908-13.
23. Lev EI, Patel RT, Guthikonda S, et al. Genetic polymorphisms of the platelet receptors P2Y (12), P2Y (1) and GPIIIa and response to aspirin and clopidogrel. *Thromb Res* 2007; 119: 355-69.
24. Gurbel PA, Bliden KP, Guyer K, et al. Delayed thrombin-induced platelet-fibrin clot generation by clopidogrel: a new dose-related effect demonstrated by thrombelastography in patients undergoing coronary artery stenting. *Thromb Res* 2007; 119: 563-70.

# Powstawanie trombiny i aktywacja płytek krwi w miejscu uszkodzenia naczyń u osób z chorobą wieńcową leczonych klopidogrelem w skojarzeniu z kwasem acetylosalicylowym

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## Streszczenie

**Wstęp:** Dane dotyczące wpływu doustnych leków przeciwpłytkowych na krzepnięcie krwi *in vivo* nie są jednoznaczne. Sugerowano, że allel PlA2 płytkowej glikoproteiny (GP) IIIa wpływa na przeciwzakrzepowe działanie klopidogrelu.

**Cel:** Zbadanie, czy stosowanie klopidogrelu w skojarzeniu z kwasem acetylosalicylowym (ASA) wpływa na produkcję trombiny i aktywację płytek krwi *in loco* pobudzone uszkodzeniem naczynia.

**Metody:** Badaniem objęto osoby ze stabilną chorobą wieńcową przewlekle przyjmujące ASA. W sposób losowy chorych przydzielano do dwóch grup: w jednej do ASA dołączano klopidogrel w dawce 75 mg dziennie (n = 30), a w drugiej kontynuowano podawanie ASA w dawce 100 mg dziennie (n = 30) przez 4 tygodnie. Markery generacji trombiny [kompleksy trombina-antytrombina (TAT) i fragmenty 1.2 protrombiny (F1.2)] oraz markery aktywacji płytek krwi [rozpuszczalny ligand CD40 (sCD40L) i selektyna P] oznaczano w nadsączu próbek krwi zbieranych w miejscu uszkodzenia mikronaczyń skóry.

**Wyniki:** Całkowita ilość trombiny, wyrażona za pomocą markerów mierzonych w miejscu uszkodzenia naczyń, była podobna przed i po dodaniu klopidogrelu do ASA. Uwalnianie sCD40L i selektyny P z płytek krwi było mniejsze w czasie terapii skojarzonej ASA i klopidogrelem odpowiednio o 33,8 i 27,8% ($p < 0,001$ dla obu porównań). Chorzy, których redukcja aktywacji płytek *in loco* mieściła się w najwyższym tercylu, charakteryzowali się przebytym zawałem serca, chorobą naczyń obwodowych i uwalnianiem największych ilości sCD40L i selektyny P przed dodaniem klopidogrelu. Generacja TAT i F1.2 oraz uwalnianie sCD40L i selektyny P nie zależały od obecności allela PlA2.

**Wnioski:** Nasze badanie pokazuje, że klopidogrel dołączony do ASA nie zmniejsza generacji trombiny mierzonej w miejscu uszkodzenia naczyń, ale znamiennie upośledza uwalnianie z płytek sCD40L i selektyny P.

**Słowa kluczowe:** klopidogrel, trombina, choroba wieńcowa, kwas acetylosalicylowy, uszkodzenie naczynia, płytki

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