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ORIGINAL ARTICLE

Effects of P2Y12 receptor inhibition with or without aspirin on hemostatic system activation: a randomized trial in healthy subjects

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Essentials

- In acute coronary syndromes, dual antiplatelet therapy inhibits platelets but confers a bleeding risk.
- Healthy male volunteers received clopidogrel or ticagrelor plus aspirin or clopidogrel or ticagrelor alone.
- The decrease in β-thromboglobulin in shed blood was comparable after single and dual antiplatelet therapy.
- We hypothesize that patients with acute coronary syndromes may not require dual antiplatelet therapy.

Summary. *Background:* Dual antiplatelet therapy with a P2Y12 inhibitor and aspirin is standard in acute coronary syndromes. Dual antiplatelet therapy causes more bleeding than single antiplatelet therapy with a P2Y12 inhibitor. *Objectives:* To compare the effects of dual and single antiplatelet therapies on hemostatic system activation. *Patients/Methods:* In a randomized, parallel-group, double-blind, placebo-controlled study, 44 healthy volunteers received clopidogrel (600 mg, then 150 mg d^{-1}) and aspirin (100 mg d^{-1}) or placebo for 7 days; An additional 44 volunteers received single-dose ticagrelor (180 mg) and aspirin (300 mg) or placebo. β-Thromboglobulin (β-TG [IU L^{-1}]) and prothrombin fragment 1.2 (f1.2 [nmol L^{-1}]) were measured in blood obtained from bleeding time incisions. Data are given as geometric mean ratio (GMR [95% confidence interval]) to describe the differences in the first 2 h and as mean differences (Δ [95% confidence interval]) in area under the curve (AUC) to discriminate differences in effects over the total observation time. *Results:* Clopidogrel plus aspirin and clopidogrel plus placebo reduced β-TG by a GMR of 0.51 (0.42–0.63) and 0.54 (0.46–0.64) at 2 h. Ticagrelor plus aspirin and ticagrelor plus placebo decreased β-TG by a GMR of 0.38 (0.26–0.57) and 0.47 (0.31–0.72). Ticagrelor plus aspirin and ticagrelor plus placebo reduced f1.2 by a GMR of 0.58 (0.45–0.75) and 0.55 (0.38–0.80); clopidogrel did not. Over 24 h, no difference in β-TG occurred between clopidogrel plus aspirin and clopidogrel plus placebo (ΔAUC = −2.9 [−9.9 to 4.1]) or between ticagrelor plus aspirin and ticagrelor plus placebo (ΔAUC = −3.5 [−11.8 to 4.7]). No difference in f1.2 occurred between clopidogrel plus aspirin and clopidogrel plus placebo (ΔAUC = −4.2 [−10.2 to 1.8]) or between ticagrelor plus aspirin and ticagrelor plus placebo (ΔAUC = −3.6 [−10.9 to 3.7]). *Conclusions:* P2Y12 inhibitor monotherapy and dual antiplatelet therapy inhibit hemostatic system activation to a comparable extent.

Keywords: acute coronary syndromes; aspirin; clopidogrel; platelet aggregation inhibitors; ticagrelor.

Introduction

Coronary heart disease is the most common cause of death in the Western world and the main reason for acute coronary syndromes [1]. Acute coronary syndromes comprise unstable angina, non–ST-segment elevation myocardial infarction, and ST-segment elevation myocardial infarction and are associated with considerable morbidity and mortality and high hospitalization rates [2,3]. Treatment of acute coronary syndromes includes revascularization by percutaneous coronary intervention, coronary artery bypass graft surgery, and thrombolysis [4]. Regarding pharmacological thromboprophylaxis, dual antiplatelet therapy consisting of aspirin and clopidogrel, a P2Y12 inhibitor, was standard clinical practice until recently. During the past years, the spectrum of P2Y12 inhibitors

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has become broader with the appearance of two new compounds: ticagrelor and prasugrel [5–7].

Although effective, dual antiplatelet therapy confers a considerable risk of bleeding regardless which of the aforementioned P2Y12 inhibitors is used. In PLATO (PLATelet inhibition and patient Outcomes), for instance, the rates of major or life-threatening/fatal bleeding at 12 months were as high as 11% and 6%, respectively, among patients treated with ticagrelor or clopidogrel in combination with aspirin [8]. Likewise, in TRITON-TIMI 38 (Trials to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis in Myocardial Infarction), applying different definitions of bleeding, non–coronary artery bypass graft surgery–related bleeding was recorded at 15 months in 2.4% and 1.8% of patients receiving prasugrel or clopidogrel together with aspirin, respectively [9].

The question of whether single antiplatelet therapy with a P2Y12 inhibitor alone is safer than dual antiplatelet therapy has so far been addressed by two trials. In MATCH (Management of Atherothrombosis With Clopidogrel in High-Risk Patients With Recent Transient Ischemic Attacks or Ischemic Stroke), single antiplatelet therapy with clopidogrel in high-risk patients with ischemic stroke or transient ischemic events resulted in lower rates of intracranial and gastrointestinal bleeding compared with dual antiplatelet therapy [10]. In WOEST (What is the Optimal antiplatElet and anticoagulant therapy in patients with oral anticoagulation and coronary StenTing), use of clopidogrel without aspirin in patients undergoing percutaneous coronary intervention was associated with a significant reduction in bleeding complications compared with patients receiving dual antiplatelet therapy [11]. So far, these studies did not alter current clinical practice. Their results, for instance, cannot be extrapolated to novel, more-potent antiplatelet agents. In WOEST, patients received oral anticoagulants in addition to the platelet inhibitors, and the study was underpowered to exclude an excess of stent thrombosis when aspirin was omitted [11]. In MATCH, patients with cerebral artery disease rather than patients with acute coronary syndromes were investigated.

Therefore, we set out to further investigate the hypothesis that single antiplatelet therapy with a P2Y12 inhibitor alone has a comparable antithrombotic effect as dual antiplatelet therapy consisting of a P2Y12 inhibitor and aspirin. Notably, we used an experimental approach that allowed us to study the effects of antiplatelet therapy on hemostatic system activation under circumstances close to the *in vivo* situation, namely in the presence of important determinants of plug formation such as blood cells, coagulation factors, endothelium, and flow.

Materials and methods

The study protocol was approved by the Ethics Committee of the Medical University of Vienna, Austria (EK 184/2010). The study was conducted according to the Declaration of Helsinki including current revisions and the ICH Good Clinical Practice guidelines. The trial is registered at clinicaltrials.gov (NCT02120092) and at the European clinical trials database (EudraCT 2010-019643-19). Written informed consent was obtained from all subjects before any study-related procedures were conducted.

Study population

The study was carried out in healthy, male, non-smoking volunteers aged 18–50 years. Exclusion criteria were history of bleeding or disorders associated with an increased bleeding risk, obesity, allergy to or contraindication against any study drug, history of or symptoms suggestive for gastrointestinal disease, any other significant finding in physical or laboratory examination, abuse of alcoholic beverages, or use of any medication within 2 weeks before blood sampling.

Study design

The study was conducted as a randomized, parallel-group, double-blind, placebo-controlled trial between November 2011 and December 2013 at the Departments of Medicine I and Clinical Pharmacology, Medical University of Vienna, Vienna, Austria. Randomization was performed by the method of permuted blocks with a block size of four. A person not directly involved in study-related procedures performed concealment of the respective drugs. Investigators involved in the study were not aware of the randomization code, which was broken after finalizing the study and all laboratory analyses were completed. The trial was carried out in two parts.

Part I (clopidogrel study) was scheduled for 8 consecutive days. Study treatment was administered in the fasting state once daily from day 1 to day 7. Blood sampling was performed on day 1 before study treatment (baseline), and then after 2, 6, and 24 h and after 8 days. On day 1, volunteers received 600 mg clopidogrel (Plavix®; Sanofi Pharma Bristol-Myers Squibb, Paris, France) together with 100 mg aspirin or 600 mg clopidogrel together with placebo followed by 150 mg clopidogrel together with 100 mg aspirin or 150 mg clopidogrel together with placebo from day 2 until day 7.

Part II (ticagrelor study) was scheduled for 2 consecutive days. Study treatment was administered in the fasting state on day 1. Blood sampling was performed on day 1 before study treatment (baseline) and after 2, 6, and 24 h. Volunteers received 180 mg ticagrelor (Brilique/Brilinta®; AstraZeneca, Södertälje, Sweden) together with 300 mg aspirin or 180 mg ticagrelor together with placebo.

Blood sampling

Bleeding time incisions were performed as described in previously performed studies by using a disposable stan-

dard device (Surgicutt®; ITC, Edison, NJ, USA) [12]. After inflation of a sphygmomanometer cuff to 40 mm Hg positioned on the upper arm, 5-mm-long and 1-mm-deep incisions positioned parallel to the antecubital crease were made on the lateral volar aspect of the forearm. The procedure was carried out by the same investigator at all times. To avoid activation of platelets and coagulation factors due to skin contact, blood (called shed blood) was collected directly from the edge of the wound over a period of 4 min using a plastic pipette (TipOne®; STARLAB Corporation, Hamburg, Germany). The blood was transferred immediately into ice-cooled plastic tubes containing an anticoagulant solution consisting of 3.8% sodium citrate, 10% aprotinin, and 0.5% indomethacin. For determination of β-thromboglobulin (β-TG), thromboxane B_2 (TxB_2), and prothrombin fragment f1.2 (f1.2) levels, tubes were centrifuged at 14 400 × *g* for 10 min at 4 °C. The clear supernatant was separated and stored at −80 °C. β-TG, f1.2, and TxB_2 concentrations in shed blood were determined by adjustment of concentrations with a correction factor calculated from the ratio of stop solution to individual shed blood volume in the collection tubes.

Assays

β-TG, f1., and TxB_2 were determined using commercially available enzyme-linked immunoassays (Asserachrom® β-TG, Stago, France; Enzygnost® F1 + 2 (monoclonal), Siemens Healthcare Diagnostics Products, Germany; Thromboxane B_2 EIA Kit, Cayman Chemical Company, Ann Arbor, MI, USA). Platelet and hemoglobin levels as well as prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen were determined using standard assays and procedures according to hospital routine.

Statistical methods

Data are given as median and quartiles. Due to their skewed distributions, continuous outcome variables were log-transformed before statistical analyses. To evaluate the short-term treatment effect, the unpaired *t*-test was used comparing baseline measurements with values after 2 hours. The differences due to aspirin were tested by comparing the changes after 2 hours between the two treatment groups (with or without aspirin) through use of the two-sample *t*-test. Effects are described by the geometric mean ratio (GMR [95% confidence interval]) obtained by retransforming mean differences from the logarithmic to the original scale. To test for differences in effects over the total observation time, the area under the curve (AUC) was calculated and compared using the two-sample *t*-test. Effects are described by the mean difference (Δ [95% confidence interval]). Separate tests were performed evaluating volunteers treated with clopidogrel (with aspirin or placebo) and volunteers treated with ticagrelor (with aspirin or placebo), respectively. All *P*-values are results of two-sided tests and *P*-values < 0.05 were considered as indicating statistical significance.

The sample size was calculated to detect a 20 percentage points difference in the percent change of the main outcome variable (β-TG in shed blood) between baseline and 2 h after treatment start (alpha: 0.05; power: 80%). The assumption of a standard deviation of 25.2 is based on prior data [13]. Following a predefined interim analysis regarding the sample size, a final sample size of 22 volunteers per group was specified.

Results

Subjects

The study population consisted of 88 healthy male volunteers (mean age 27 years), of whom 44 participated in the clopidogrel study and 44 in the ticagrelor study. A severe adverse event was encountered in none of the volunteers. One volunteer had nose bleeding for ~1 min and a hematoma on the thigh ~7 cm in diameter. He was treated with clopidogrel and aspirin, reported to the study center on day 8, completed treatment, and did not receive a specific therapy.

Table 1 Effects of clopidogrel combined with aspirin and clopidogrel combined with placebo on β-TG, f1.2, and TxB_2 levels in shed blood from baseline to 2 h in 44 healthy male volunteers

	β-TG (IU L^{-1})		f1.2 (nmol L^{-1})		TxB_2 (ng mL^{-1})	
	Clopidogrel + aspirin	Clopidogrel + placebo	Clopidogrel + aspirin	Clopidogrel + placebo	Clopidogrel + aspirin	Clopidogrel + placebo
Baseline	2.6 (2.3–4.5)	2.6 (1.8–3.4)	6.1 (4.7–8.9)	6.4 (4.5–7.6)	15.2 (7.3–19.8)	13.8 (7.8–19.8)
2 h	1.5 (1.1–2.2)	1.1 (0.9–2.2)	5.8 (4.4–6.8)	6.2 (4.3–7.3)	2.1 (1.1–4.9)	10.7 (5.1–13.6)
P-value*	< 0.001	< 0.001	0.47	0.43	< 0.001	0.02
P-value†	0.71		0.93		< 0.001	
GMR*	0.51 (0.42–0.63)	0.54 (0.46–0.64)	0.91 (0.68–1.2)	0.92 (0,74–1.14)	0.18 (0.12–0.28)	0.71 (0.54–0.93)
GMR†	0.95 (0.73–1.23)		0.98 (0.69–1.4)		0.26 (0.16–0.42)	

β-TG, β-thromboglobulin; fl.2, prothrombin fragment 1.2; TxB_2, thromboxane B_2.
*From baseline to 2 h. †Changes between treatment groups; values are given as median (quartiles) and GMR (95% confidence interval).

Effects of clopidogrel and aspirin and of clopidogrel and placebo on hemostatic system activation in the microcirculation

Compared with baseline, a significant decrease in the generation of β-TG was found at 2 h both in volunteers receiving clopidogrel and aspirin and in volunteers given clopidogrel and placebo, as represented by reductions in β-TG shed blood levels by a GMR of 0.51 (95% confidence interval 0.42–0.63) and 0.54 (0.46–0.64), respectively. No statistical difference in the extent of decrease was observed between the two groups (Table 1; Fig. 1, Panel A). Regarding f1.2, no statistically significant difference between baseline and 2 h was found either within or between groups (Table 1; Fig. 1, Panel B). Compared with baseline, both treatment with clopidogrel and aspirin and treatment with clopidogrel and placebo significantly reduced the generation of TxB_2 at 2 h. This effect was more pronounced among volunteers receiving clopidogrel and aspirin (GMR = 0.26 [0.16–0.42]) (Table 1; Fig. 1, Panel C). Regarding β-TG, there was no significant difference in the median AUC from 2 to 24 h (AUC_{h24}) and from 2 h to 8 days (AUC_{d8}) between volunteers treated with clopidogrel and aspirin and volunteers given clopidogrel and placebo (Table 2). There was no significant difference in the f1.2 generation between volunteers treated with clopidogrel and aspirin and volunteers given clopidogrel and placebo from 2 to 24 h (AUC_{h24}). From 2 h to 8 days (AUC_{d8}), generation of f1.2 was less pronounced in volunteers receiving clopidogrel and aspirin compared with those treated with clopidogrel and placebo (Table 2). Both from 2 to 24 h (AUC_{h24}) and from 2 h to 8 days (AUC_{d8}), volunteers treated with clopidogrel and aspirin experienced a significantly more pronounced reduction in TxB_2 formation than did volunteers treated with clopidogrel and placebo (Table 2).

Effects of ticagrelor and aspirin and of ticagrelor and placebo on hemostatic system activation in the microcirculation

Compared with baseline, a significant reduction in the generation of β-TG was found at 2 h both in volunteers receiving ticagrelor and aspirin and in volunteers given ticagrelor and placebo, as indicated by reductions in β-TG shed blood levels by a GMR of 0.38 (0.26–0.57) and 0.47 (0.31–0.72), respectively. No statistical difference was observed between the two groups (Table 3; Fig. 2, Panel A). Both ticagrelor and aspirin and ticagrelor and placebo caused a substantial reduction in the generation of f1.2 at 2 h compared with baseline, but there was no difference between the two groups regarding the extent of this effect (Table 3; Fig. 2, Panel B). Ticagrelor and aspirin and ticagrelor and placebo both significantly reduced the generation of TxB_2 after 2 h with a more pronounced effect of ticagrelor combined with aspirin

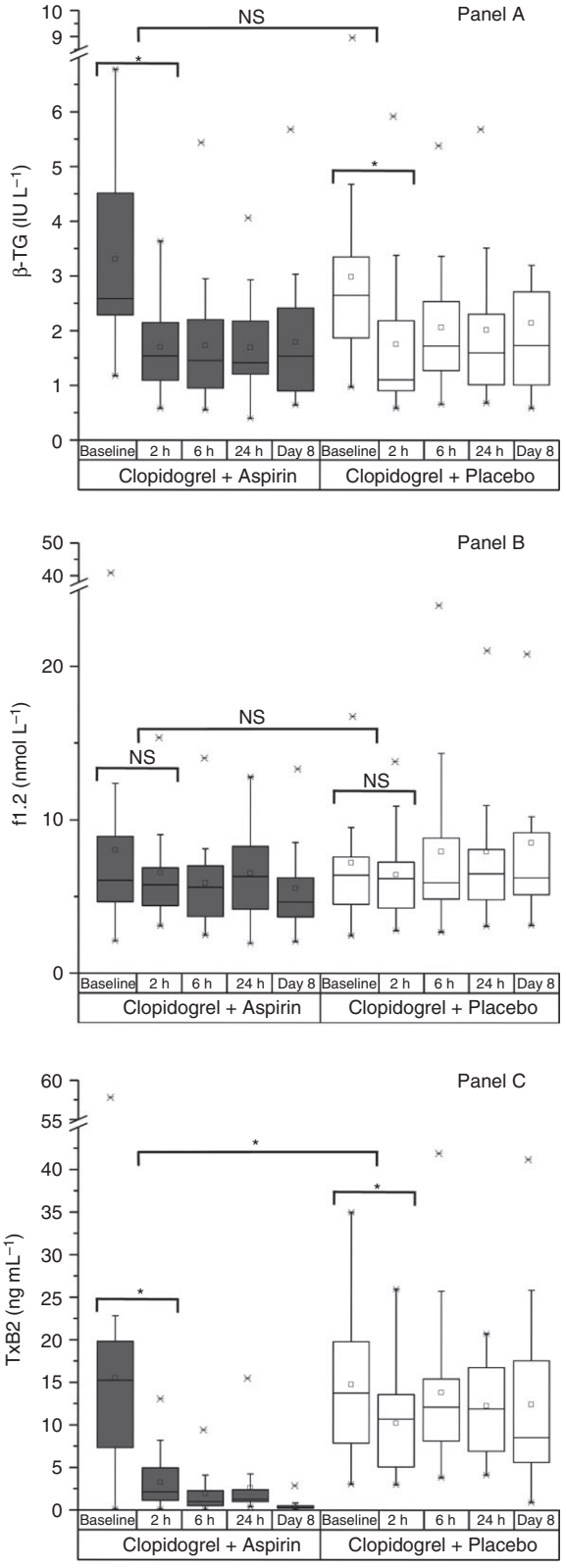

Fig. 1. Boxplots demonstrating the effects of clopidogrel and aspirin and clopidogrel and placebo on β-TG (Panel A), f1.2 (Panel B) and TxB2 (Panel C) levels in shed blood at different time points over the observational time compared with baseline levels. $*P < 0.05$ for changes from baseline to 2 h and between treatment groups. NS, non significant.

Table 2 Effects of clopidogrel combined with aspirin and clopidogrel combined with placebo on hemostatic system activation as represented by the area under the corresponding concentration curve from 2 to 24 h (AUC_{h24}) and from 2 hours to 8 days (AUC_{8d}) in 44 healthy male volunteers

	β-TG		f1.2		TxB2	
	Clopidogrel + aspirin	Clopidogrel + placebo	Clopidogrel + aspirin	Clopidogrel + placebo	Clopidogrel + aspirin	Clopidogrel + placebo
AUC_{h24}	161 (156–168)	162 (154–173)	189 (182–196)	191 (188–200)	159 (146–173)	204 (194–215)
P-$value_{h24}$	0.4		0.16		< 0.001	
ΔAUC_{h24}	−2.9 (−9.9 to 4.1)		−4.2 (−10.2 to 1.8)		−46.0 (−56.5 to −35.6)	
AUC_{8d}	1390 (1337–1448)	1379 (1326–1467)	1423 (1380–1471)	1456 (1428–1504)	1106 (1043–1175)	1501 (1465–1609)
P-$value_{8d}$	0.4		0.04		< 0.001	
ΔAUC_{8d}	−26.0 (−88.5 to 36.6)		−47.0 (−91.1 to −2.8)		−418.0 (−486.2 to −349.8)	

β-TG, β-thromboglobulin; fl.2, prothrombin fragment 1.2; TxB2, thromboxane B2.
AUC calculation was based on log-transformed concentrations; values are given as median (quartiles) and ΔAUC (95% confidence interval).

Table 3 Effects of ticagrelor combined with aspirin and ticagrelor combined with placebo on β-TG, f1.2, and TxB_2 levels in shed blood from baseline to 2 h in 44 healthy male volunteers

	β-TG (IU L^{-1})		f1.2(nmol L^{-1})		TxB2 (ng mL^{-1})	
	Ticagrelor + aspirin	Ticagrelor + placebo	Ticagrelor + aspirin	Ticagrelor + placebo	Ticagrelor + aspirin	Ticagrelor + placebo
Baseline	1.7 (1.2–2.9)	1.8 (1.3–2.6)	10.1 (7.1–20.2)	9.8 (8.7–17.8)	24.0 (12.4–37.7)	23.8 (12.1–30.9)
2 h	0.7 (0.5–1.2)	0.8 (0.6–1.3)	6.1 (3.8–10.3)	6.9 (4.9–9.5)	0.6 (0.4–0.9)	8.3 (3.9–14.5)
P-value*	< 0.001	0.001	< 0.001	0.003	< 0.001	< 0.001
P-value†	0.5		0.8		< 0.001	
GMR*	0.38 (0.26–0.57)	0.47 (0.31–0.72)	0.58 (0.45–0.75)	0.55 (0.38–0.8)	0.025 (0.015–0.04)	0.37 (0.25–0.54)
GMR†	0.81 (0.46–1.44)		1.06 (0.69–1.65)		0.07 (0.04–0.12)	

β-TG, β-thromboglobulin; fl.2, prothrombin fragment 1.2; TxB2, thromboxane B2.
*From baseline to 2 h. †Changes between treatment groups; values are given as median (quartiles) and GMR (95% confidence interval).

than of ticagrelor and placebo (GMR = 0.07 [0.04–0.12]) (Table 3; Fig. 2, Panel C). There was no significant difference in the median AUC values calculated from 2 to 24 h between volunteers treated with ticagrelor and aspirin compared with volunteers given ticagrelor and placebo for either β-TG or f1.2 (Table 4). From 2 to 24 h, volunteers treated with ticagrelor and aspirin experienced a more-pronounced reduction in TxB_2 formation than did volunteers treated with ticagrelor and placebo (Table 4).

Discussion

The principal finding of our study was that in healthy male volunteers single antiplatelet therapy with clopidogrel or ticagrelor inhibited platelet activation in the microcirculation (as represented by the concentration of β-TG in shed blood) to a similar extent as dual antiplatelet therapy consisting of clopidogrel or ticagrelor combined with aspirin. We also found that the activation of the coagulation cascade (as represented by the level of f1.2 in shed blood) was significantly more reduced by ticagrelor than by clopidogrel regardless of whether aspirin was coadministered. Microvascular TxB_2 generation was substantially affected when aspirin was combined with clopidogrel or ticagrelor but was also inhibited by clopidogrel or ticagrelor monotherapy.

In patients with acute coronary syndromes, dual antiplatelet therapy is currently the cornerstone of antithrombotic treatment and its use is recommended by guideline panels [4–7]. Dual antiplatelet therapy is considered to be effective, but there is evidence from many clinical trials that dual antiplatelet therapy confers a substantial bleeding risk [8–11,14–16]. Moreover, patients who have a bleeding complication and must discontinue dual antiplatelet therapy are at high risk of a subsequent vascular event [17,18]. It is therefore of clinical importance to test the concept that single antiplatelet therapy with a P2Y12 inhibitor alone confers a lower risk of bleeding than dual antiplatelet therapy but, most importantly, at the same time also reduces the risk of thrombosis to a comparable extent. Our findings of a similar inhibition of hemostatic system activation by single antiplatelet therapy and dual antiplatelet therapy support this notion. We found that single antiplatelet therapy (consisting of clopidogrel or ticagrelor alone) and dual antiplatelet therapy (consisting of clopidogrel or ticagrelor together with aspirin) suppressed platelet reactivity by 40% to 60%. Importantly, there was no difference in the extent of platelet inhibition

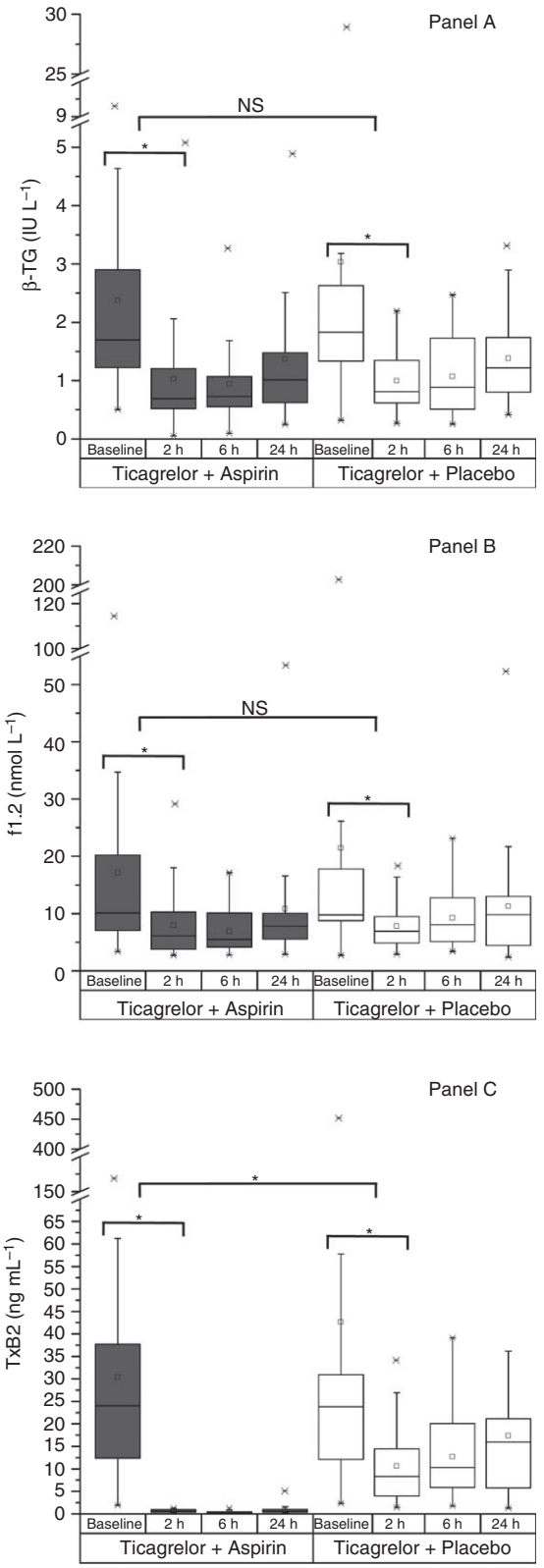

Fig. 2. Boxplots demonstrating the effects of ticagrelor and aspirin and ticagrelor and placebo on β-TG (Panel A), f1.2 (Panel B) and TxB2 (Panel C) levels in shed blood at different time points over the observational time compared with baseline levels. $*P < 0.05$ for changes from baseline to 2 h and between treatment groups. NS, non significant.

between single antiplatelet therapy and dual antiplatelet therapy, neither at 2 h nor over a longer observation period. This finding is in line with the results of two clinical trials: MATCH was a randomized, double-blind, placebo-controlled trial to compare aspirin 75 mg d^{-1} with placebo in > 7500 high-risk patients with ischemic stroke or transient ischemic attack who were already receiving clopidogrel 75 mg d^{-1}. The primary study end point, a composite of ischemic stroke, myocardial infarction, vascular death, or rehospitalization for acute ischemia, was seen in 15.7% patients in the group receiving aspirin and clopidogrel compared with 16.7% in the clopidogrel-alone group for a statistically non-significant relative risk reduction of 6.4%. WOEST was an open-label, multicenter, randomized, controlled trial to compare clopidogrel alone and clopidogrel plus aspirin in 573 patients receiving oral anticoagulant treatment and undergoing percutaneous coronary intervention. The efficacy end point, a composite of death, myocardial infarction, stroke, target-vessel revascularization, and stent thrombosis, occurred in 11.1% of patients receiving single antiplatelet therapy and in 17.6% of patients given dual antiplatelet therapy for a statistically significant risk reduction of 40%. Regarding safety, in both studies bleeding was less frequently seen among patients receiving single antiplatelet therapy. In MATCH, life-threatening bleeding (including intracranial and gastrointestinal bleeding) and major and minor bleeding were recorded significantly more often in patients with dual antiplatelet therapy than in those with clopidogrel alone. In WOEST, the primary study end point, any bleeding, was recorded in 19.4% of patients receiving clopidogrel on top of oral anticoagulation compared with 44.4% of patients receiving triple therapy with aspirin [10,11]. Thus, the results of the aforementioned studies together with our *in vivo* experimental data point toward a more favorable balance between thrombosis prevention and risk of bleeding for single antiplatelet therapy with a P2Y12 inhibitor alone compared with dual antiplatelet therapy.

We made two other interesting observations. First, we found that ticagrelor given alone or in combination with aspirin inhibited activation of the coagulation system (as represented by an ~40% reduction of f1.2 in shed blood), whereas no such effect was seen with clopidogrel. One may speculate that the anticoagulant effect of ticagrelor is, at least in part, responsible for the improved efficacy of ticagrelor compared with clopidogrel as recently reported by the PLATO investigators [8]. Second, as expected and demonstrated previously [12,19], TxB_2 generation in the microcirculation was almost completely blunted by aspirin regardless of which P2Y12 inhibitor we combined with aspirin. Interestingly, both clopidogrel and ticagrelor, when given as monotherapy, also significantly decreased the formation of TxB_2 in the microcirculation. This observation confirms and extends the findings of Armstrong *et al.* [20], who reported an almost 60%

Table 4 Effects of ticagrelor combined with aspirin and ticagrelor combined with placebo on hemostatic system activation as represented by the area under the corresponding concentration curve from 2 to 24 h (AUC_{h24}) in 44 healthy male volunteers

	β-TG		f1.2		TxB2	
	Ticagrelor + aspirin	Ticagrelor + placebo	Ticagrelor + aspirin	Ticagrelor + placebo	Ticagrelor + aspirin	Ticagrelor + placebo
AUC_{h24}	146 (139–157)	153 (142–161)	192 (187–201)	198 (188–207)	133 (128–143)	207 (189–217)
P-$value_{h24}$	0.4		0.3		< 0.001	
ΔAUC_{h24}	−3.5 (−11.8 to 4.7)		−3.6 (−10.9 to 3.7)		−70.9 (−81.0 to −60.8)	

β-TG, β-thromboglobulin; fl.2, prothrombin fragment 1.2; TxB2, thromboxane B2.
AUC calculation was based on log-transformed concentrations; values are given as median (quartiles) and ΔAUC (95% confidence interval).

reduction of urinary 11-dh-TXB_2 levels in healthy volunteers given either clopidogrel or aspirin. In their study, clopidogrel caused a significant reduction in the production of TxB_2 in platelet-rich plasma in response to collagen, arachidonic acid, or epinephrine. It is, therefore, obvious that activation of the P2Y12 receptor causes production of thromboxane, which can be inhibited not only by aspirin but also by ticagrelor or by clopidogrel, although, according to our data, to a lesser extent.

Our work has strengths and limitations. In patients with acute coronary syndromes, thrombus formation is localized to the coronary injury site and, as a consequence, laboratory studies performed in venous or arterial blood may not truly cover all the mechanisms leading to coagulation and platelet activation relevant under *in vivo* circumstances. To overcome this limitation, we applied a technique, which consists of measuring coagulation and platelet activation indicators in blood emerging from a standardized local microvascular injury made to determine bleeding time. Using this method, we were able to study hemostatic system activation *in vivo* in the presence of important determinants of plug formation such as blood cells, coagulation factors, endothelium, and flow. Of note, this method has already been successfully used to study various antithrombotic agents under *in vivo* circumstances [13,21–25]. Nevertheless, it has to be considered that shear rate conditions in stenosed or stented coronary arteries are different from those in the microvasculature. Considering the complexity of our experimental approach, a large number of individuals were included. The design of the study was robust as it was carried out in a randomized, parallel-group, double-blind, placebo-controlled manner. One important limitation is that, for practical reasons and to avoid discontinuation of conventional treatment, our experiments were performed in volunteers rather than in patients with acute coronary syndromes, who are likely to be older and in whom hemostatic system activation may be more pronounced. In addition, for ethical reasons, we kept the treatment period as short as possible: 7 days in the clopidogrel study and only one dose in the ticagrelor study. We, therefore, cannot comment on the long-term effects associated with the antiplatelet regimen tested. We did not use a loading dose for aspirin in the clopidogrel study because of safety considerations, and we increased the dose to 300 mg in the ticagrelor study only after having seen minimal bleeding in the previous study. For logistic and financial reasons, the study was carried out in two parts with an interim of ~1 year between the clopidogrel and the ticagrelor study. This could explain the differences in baseline values between the two studies as subject characteristic and, more importantly, analytical conditions may not have been exactly the same. Because of the aforementioned methodological limitations, this study has to be regarded as hypothesis-generating and more research is required to appreciate the relevance of our findings for clinical practice.

In conclusion, using an *in vivo* experimental approach, we found no difference in the inhibition of platelet activation between single antiplatelet therapy with a P2Y12 inhibitor (clopidogrel or ticagrelor) alone and dual antiplatelet therapy. Our results support the concept that patients with acute coronary syndromes no longer require dual antiplatelet therapy because single antiplatelet therapy with a P2Y12 inhibitor alone inhibits hemostatic system activation to a similar extent.

Addendum

P. A. Kyrle and L. Traby drafted the manuscript. A. Kaider and L. Traby performed statistical analysis and interpreted data. M. Kollars was responsible for acquisition, laboratory analysis, and interpretation of data. P. A. Kyrle, S. Eichinger and M. Wolzt were responsible for the concept and design of the study. S. Eichinger and M. Wolzt critically revised the manuscript for important intellectual content.

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Disclosure of Conflict of interests

S. Eichinger reports personal fees from Bayer, Boehringer-Ingelheim, Daiichi-Sankyo, and Pfizer, outside the

submitted work. The other authors state that they have no conflict of interest.

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