Effects of prasugrel on platelet inhibition during systemic endotoxaemia: a randomized controlled trial

Alexander O. SPIEL*†, Ulla DERHASCHNIG*†, Michael SCHWAMEIS*, Johann BARTKO*, Jolanta M. SILLER-MATULA*‡ and Bernd JILMA*

*Department of Clinical Pharmacology, Medical University of Vienna, Vienna, Austria, †Department of Emergency Medicine, Medical University of Vienna, Vienna, Austria, and ‡Department of Cardiology, Medical University of Vienna, Vienna, Austria

ABSTRACT

P2Y₁₂ receptor antagonists have become a mainstay for the treatment of CVD (cardiovascular diseases). However, they have rarely been evaluated under pathophysiological conditions apart from arterial diseases. We hypothesized interactions between prasugrel and enhanced vWF (von Willebrand Factor) release in a model of systemic inflammation, and compared the pharmacodynamic effects of prasugrel against placebo on agonist-induced platelet aggregation and shear-induced platelet plug formation. A total of 20 healthy male volunteers were enrolled in a double-blind placebo-controlled two-way crossover trial. Each volunteer received either placebo or a 60 mg loading dose of prasugrel 2 h before endotoxin or placebo infusion. Platelet inhibition was measured with MEA (multiple electrode aggregometry), the PFA-100 system and the VASP (vasodilator-stimulated phosphoprotein) phosphorylation assay. Prasugrel blunted various platelet aggregation pathways, including those induced by ADP (−81%), AA (arachidonic acid) (−60%), ristocetin (−75%; P < 0.001 for all) and, to a lesser degree, collagen or TRAP (thrombin-receptor-activating peptide). Prasugrel decreased shear-induced platelet plug formation, but vWF release during endotoxaemia partly antagonized the inhibitory effect of prasugrel as measured with the PFA-100 system. Endotoxaemia acutely decreased ristocetin and TRAP-induced platelet aggregation, and enhanced ristocetin-induced aggregation after 24 h. Strong in vivo blockade of P2Y₁₂ inhibits a broad spectrum of platelet aggregation pathways. However, vWF release may reduce prasugrel’s effects under high-shear conditions.

INTRODUCTION

The P2Y₁₂ receptor plays a crucial role in thrombus formation and stabilization. Stimulation of P2Y₁₂ promotes release of the platelet agonist ADP from dense granules [1], is essential for ADP-mediated complete activation of glycoprotein IIb/IIIa and Ia/IIa, and further stabilizes platelet aggregates [2]. The P2Y₁₂ receptor antagonists ticlopidine, clopidogrel and prasugrel irreversibly inhibit the P2Y₁₂ receptor [3]. Consistent with the central role of the P2Y₁₂ receptor in thrombosis, P2Y₁₂ receptor antagonists have become a mainstay for the treatment of CVD (cardiovascular diseases). However, they have rarely been evaluated under pathophysiological conditions apart from arterial diseases. We hypothesized interactions between prasugrel and enhanced vWF (von Willebrand Factor) release in a model of systemic inflammation, and compared the pharmacodynamic effects of prasugrel against placebo on agonist-induced platelet aggregation and shear-induced platelet plug formation. A total of 20 healthy male volunteers were enrolled in a double-blind placebo-controlled two-way crossover trial. Each volunteer received either placebo or a 60 mg loading dose of prasugrel 2 h before endotoxin or placebo infusion. Platelet inhibition was measured with MEA (multiple electrode aggregometry), the PFA-100 system and the VASP (vasodilator-stimulated phosphoprotein) phosphorylation assay. Prasugrel blunted various platelet aggregation pathways, including those induced by ADP (−81%), AA (arachidonic acid) (−60%), ristocetin (−75%; P < 0.001 for all) and, to a lesser degree, collagen or TRAP (thrombin-receptor-activating peptide). Prasugrel decreased shear-induced platelet plug formation, but vWF release during endotoxaemia partly antagonized the inhibitory effect of prasugrel as measured with the PFA-100 system. Endotoxaemia acutely decreased ristocetin and TRAP-induced platelet aggregation, and enhanced ristocetin-induced aggregation after 24 h. Strong in vivo blockade of P2Y₁₂ inhibits a broad spectrum of platelet aggregation pathways. However, vWF release may reduce prasugrel’s effects under high-shear conditions.

Key words: aggregation, human endotoxaemia, platelet, prasugrel, randomized controlled trial.

Abbreviations: AA, arachidonic acid; AUC, area under the curve; BMI, body mass index; CAD, coronary artery disease; CADP, collagen/ADP; CEPI, collagen/adrenaline (epinephrine); CI, confidence interval; CT, closure time; CVD, cardiovascular disease; GMFI, geometric mean fluorescence intensity; IQR, interquartile range; LPS, lipopolysaccharide; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; PAR1, protease-activated receptor 1; PGE₁, prostaglandin E₁; PRI, platelet reactivity index; TRAP, thrombin-receptor-activating peptide; TXA₂, thromboxane A₂; VASP, vasodilator-stimulated phosphoprotein; vWF, von Willebrand factor; vWF:Ag, vWF antigen.

Correspondence: Dr Bernd Jilma (email Bernd.Jilma@meduniwien.ac.at).
of patients with acute coronary syndromes, particularly after PCI (percutaneous coronary intervention) and stenting, and significant cerebrovascular and peripheral artery diseases [4,5].

However, P2Y_{12} receptor antagonists have rarely been evaluated under pathophysiological conditions apart from CVD (cardiovascular diseases). Only recently has an association between aspirin use and lower mortality been reported in critically ill patients [6]. Human low-dose endotoxaemia is a well-standardized model of systemic inflammation [7] which induces release of vWF (von Willebrand factor) [8]. Little is known about changes in platelet function induced by endotoxaemia, which causes a small fall in platelet counts [9]. Platelet activation has been demonstrated by increases in the platelet activation marker soluble P-selectin, which could be partly mediated by thrombin [10]. The response to TRAP (thrombin-receptor-activating peptide) decreases due to PAR1 (protease-activated receptor 1) down-regulation [11]. In contrast, platelet plug formation under high shear rates is enhanced, which is at least partly due to vWF release [8].

We hypothesized that strong P2Y_{12} inhibition by prasugrel may on the one hand decrease platelet plug formation under high shear conditions, and that vWF on the other hand could reduce the effects of prasugrel. In addition, we set out to further characterize the changes in platelet aggregation during endotoxaemia in a randomized placebo-controlled trial. Shear-induced platelet plug formation was quantified using the PFA-100 system (Siemens Healthcare Diagnostics), and agonist-induced platelet aggregation was measured with whole-blood aggregometry (multiplate MEA (multiple electrode aggregometry); Dynabyte Medical), both of which have been found useful for the prediction of future events in patients with CVD [12–16].

**MATERIALS AND METHODS**

**Study design**

Written informed consent was obtained from all study participants before study entry. The Ethics Committee of the Medical University of Vienna approved the study protocol and the trial was conducted in accordance with the Declaration of Helsinki and registered at ClinicalTrials.gov as NCT01099566. The trial was block-randomized (n = 20), double blind placebo-controlled and two-way crossover. Each volunteer received two treatments consisting of a standard oral loading dose of 60 mg of prasugrel (Efient; Eli Lilly) and placebo. Prasugrel was used because it provides more consistent and stronger inhibition of P2Y_{12} than clopidogrel with much less intersubject variability [17]. The two study periods were separated by a wash-out period of at least 6 weeks between study days. In parallel, a group consisting of four subjects received prasugrel or placebo only (without LPS (lipopolysaccharide)) and served as controls. Randomization was performed using sealed opaque envelopes, which were produced before the start of the study by an independent biostatistician not otherwise associated with the study. A pharmacist, similarly not involved in the trial, encapsulated the pills consisting of lactose/starch. These pills were administered as placebo. Prasugrel tablets were encapsulated likewise. In this way, both, the study staff involved in subjects’ care or analysis of blood samples and the subjects themselves were not aware of the administered treatment.

Medical screening included medical history, physical examination, laboratory parameters, virology and standard drug screening, and was unremarkable in all study subjects. Exclusion criteria were regular or recent intake of medication including OTC (over-the-counter) drugs and clinically relevant abnormal findings in medical history or laboratory parameters. Volunteers reported to the study ward at 08:00 h after an overnight fast and were randomized to receive the trial drug (six capsculated prasugrel tablets or six placebo tablets) after baseline blood drawing (−2 h). After 2 h (0 h), volunteers received an intravenous bolus of LPS (2 ng/kg of body weight; National Reference Endotoxin, *Escherichia coli*, CC-RE-Lot 2, National Institutes of Health). Detailed study procedures of the LPS model have been outlined previously [18,19]. Throughout the first 8 h after LPS infusion, all subjects were confined to bed rest, and vital parameters were monitored on an automated monitoring system (Care View System; Hewlett Packard). Concurrently, physiological saline (200 ml/h) was administered to all subjects to maintain adequate hydration. Thereafter, study subjects were allowed to leave the ward and then returned the next morning for the 24-h blood drawing. All study procedures for the second study period were the same, except for the alternative study treatment. Paracetamol was used to alleviate flu-like symptoms if needed [20].

**Blood sampling**

Blood samples were collected from an antecubital vein by venipuncture prior to trial drug administration (−2 h), shortly before (0 h), and then at 2, 4, 6 and 24 h after LPS infusion. Measurements were performed within 1 h after blood sampling.

**Materials**

Tubes with 3.8% citrate (BD Vacutainer; Becton Dickinson), 3.2% citrate (BD Vacutainer), EDTA anticoagulant (BD Vacutainer) and recombinant hirudin (200 units/ml; Dynabyte Medical) were used for blood collection. ADP, AA (arachidonic acid), collagen, ristocetin and TRAP6 were obtained from Dynabyte Medical. CEPI [collagen/adrenaline (epinephrine)], CADP (collagen/ADP) and INNOVANCE® PFA P2Y
(only ten subjects were tested with this cartridge because it became available after the study began) cartridges were purchased from Siemens Healthcare Diagnostics.

**Platelet count**
Platelet counts were quantified with a cell counter (XE-2100; Sysmex).

**Analysis of VASP (vasodilator-stimulated phosphoprotein) phosphorylation by flow cytometry**
To determine the VASP phosphorylation state of whole blood, we used a standardized flow cytometric assay (Platelet VASP; BioCytex). Blood samples collected in 3.8 % sodium citrate were incubated in vitro with ADP and/or PGE1 (prostaglandin E1) before fixation, according to the manufacturer's instructions. After 10 min, platelets were permeabilized, labelled with a primary monoclonal antibody against Ser239-phosphorylated VASP (clone 16C2) or its isotope, followed by a secondary FITC-conjugated polyclonal goat anti-mouse antibody. All procedures were performed at room temperature (22 °C). GMFI (geometric mean fluorescence intensity) was determined using a flow cytometer (FACSCalibur™ System; BD Biosciences). The platelet population was identified by its forward and side scatter distribution, and 10 000 platelet events were gated and analysed for GMFI. Platelet reactivity was expressed as the PRI (platelet reactivity index), calculated as PRI% = [(GMFI (PGE1)–GMFI (PGE1 + ADP))/GMFI (PGE1)]×100. The ratio is expressed as the mean percentage of platelet reactivity. The normal value of the PRI is 69–100 % [21,22]. The VASP assay has been shown to have a high reproducibility, even after repeated testing of the same sample over 24 h. This was reproducible in our laboratory; the coefficient of variation for duplicate analysis was 5 % [22].

**Whole-blood impedance aggregometry: MEA**
Whole-blood aggregation was determined using MEA on a new-generation impedance aggregometer (Multiplate Analyser; Dynabyte Medical) [23]. The system detects the electrical impedance change due to the adhesion and aggregation of platelets on two independent electrode-set surfaces in the test cuvette. The AUC (area under the curve) expresses the aggregation response over the measured time [AU (aggregation units) × min], which can predict adverse outcome of patients and even bleeding [24]. A number of specific multiplate test reagents are available for the detection of different receptors/signal transduction pathways (AA, thrombin receptor, ADP receptor, collagen receptor and glycoprotein Ib-IX) in platelet activation. Whole blood was anti-coagulated with hirudin (200 units/ml; Dynabyte), as recommended by the manufacturer, and stored at room temperature (22 °C) for 30 min. Afterwards, 1:2 mixtures of 0.9 % NaCl and hirudin-anti-coagulated blood were stirred at 37 °C for 3 min in the test cuvettes. Thereafter, ADP (6.4 μM), collagen (3.2 μg), AA (0.5 mM), ristocetin (0.77 mg/ml) and TRAP6 (32 μM) were added to separate samples at concentrations recommended for MEA and the increase in electrical impedance was recorded continuously for 6 min. The mean values of the two independent determinations were expressed as the AUC of the aggregation tracing. Normal values have recently been published [25]. The MEA instrument allows two ways to express the AUC: as arbitrary aggregation units (AU × min) or as units (10 AU × min correspond to 1 unit). We measured the AUC in units, following the current recommendation of the manufacturer and many recent studies [16,26].

**Measurement of shear-induced platelet function and vWF antigen concentration**
The FDA (Food and Drug Administration)-approved platelet function analyser (PFA-100 system; Siemens Healthcare Diagnostics) has been described in detail previously [27–29]. In brief, the system consists of a disposable test cartridge where a platelet plug occludes a microscopic aperture cut into a membrane coated with collagen and either CEPI or CADP. The plug formation occurs under high shear flow conditions produced by a constant vacuum and controlled by a capillary. The time required for occlusion (CT, closure time) is indicative of platelet function and primary haemostasis capacity. The INNOVANCE® P2Y cartridge was designed to be sensitive to P2Y12 inhibition [30] and to minimize the interference of vWF.

The vWF:Ag (vWF antigen) concentration was measured with a fully automated simultaneous thermal analyser using the STA Liatest vWF (Diagnostica Stago) [31,32].

**Sample size estimation and statistical analysis**
A formal sample size calculation was not possible because of the lack of data on the magnitude of the vWF–prasugrel interaction, but five subjects would have been sufficient to demonstrate inhibition of ADP-induced aggregation by prasugrel. A sample size of 16 subjects was chosen for LPS treatment to allow the detection of a 1 S.D. (15 %) change in CADP-CT due to vWF release in the prasugrel period. No formal statistical tests were performed for control groups without LPS. All data are expressed as medians and IQR (interquartile range), or means together with the 95 % CI (confidence interval) or range, unless otherwise stated. A repeated-measures ANOVA was used for analysis of treatment and period effects (treatment, independent factor; period,
Table 1  Baseline values at the beginning of the study periods

Values are medians (interquartile range). COL, collagen; RISTO, ristocetin.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Prasugrel period</th>
<th>Placebo period</th>
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</thead>
<tbody>
<tr>
<td>VASP-PRI (%)</td>
<td>+ LPS (n = 16)</td>
<td>− LPS (n = 4)</td>
</tr>
<tr>
<td></td>
<td>81 (74–85)</td>
<td>83 (71–84)</td>
</tr>
<tr>
<td>MEA:AA (units)</td>
<td>78 (70–85)</td>
<td>60 (53–67)</td>
</tr>
<tr>
<td>MEA:ADP (units)</td>
<td>54 (48–63)</td>
<td>46 (43–49)</td>
</tr>
<tr>
<td>MEA:COL (units)</td>
<td>67 (79–125)</td>
<td>67 (47–82)</td>
</tr>
<tr>
<td>MEA:RISTO (units)</td>
<td>97 (79–125)</td>
<td>69 (60–74)</td>
</tr>
<tr>
<td>MEA:TRAP (units)</td>
<td>90 (83–105)</td>
<td>93 (75–105)</td>
</tr>
<tr>
<td>CADP-CT (s)</td>
<td>113 (99–128)</td>
<td>122 (101–215)</td>
</tr>
<tr>
<td>CEPI-CT (s)</td>
<td>144 (123–156)</td>
<td>167 (135–247)</td>
</tr>
<tr>
<td>Platelet count (×10⁹/l)</td>
<td>203 (181–256)</td>
<td>180 (165–222)</td>
</tr>
<tr>
<td>Absolute neutrophil count (×10⁹/l)</td>
<td>2.6 (2.1–3.4)</td>
<td>2.5 (1.6–3.5)</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>14.6 (14.2–14.8)</td>
<td>14.5 (13.2–16.0)</td>
</tr>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>0.05 (0.02–0.11)</td>
<td>0.03 (0.01–0.11)</td>
</tr>
</tbody>
</table>

independent factor; and outcome variable, dependent factor). When significant, post-hoc comparisons were performed with non-parametric tests for reasons of robustness: the Friedman ANOVA and the Wilcoxon test for post-hoc comparisons were used. To avoid multiple testing, we limited statistical comparisons to time points of peak effects known from previous studies. A two-tailed P < 0.05 was considered significant. All statistical calculations were performed using commercially available statistical software (Statistica version 6.1; Stat Soft).

RESULTS

Subject flow and demographic characteristics

From a total of 23 screened subjects, three subjects were not eligible for randomization into this trial (inclusion/exclusion criteria). The remaining 20 healthy male subjects were enrolled into this trial conducted between mid-October 2009 and the end of September 2010. A total of 16 subjects received two treatments consisting of 60 mg of prasugrel or placebo orally, followed by an intravenous bolus of LPS (2 ng/kg of body weight), four subjects received prasugrel or placebo only (without LPS) and served as controls. Data from all subjects was included in each analysis. The subjects in the LPS group had a mean age of 29 (range, 22–39) years and a mean BMI (body mass index) of 23 (range, 19–28) kg/m². Age [mean, 31 (range, 22–37) years] and BMI [mean, 23 (range, 22–24) kg/m²] were comparable in the control group without LPS. Although common and known adverse effects of endotoxaemia such as flu-like symptoms occurred, there were no relevant adverse events or new safety concerns or adverse effects associated with the administration of prasugrel or LPS in any study subject. The baseline values at the beginning of both study periods are presented in Table 1. No statistically significant difference was observed between periods and groups. Platelet counts significantly fell by 11–15% after LPS infusion, with no difference between treatment periods (Figure 1).

VASP phosphorylation shows a rapid and consistent antiplatelet effect of prasugrel

The PRI decreased from 80% (95% CI, 75–84%) to 5% (95% CI, 3–8%; P < 0.0001 compared with placebo) 2 h after prasugrel loading and stayed at that level for 24 h. Endotoxaemia did not affect VASP phosphorylation (Figure 1).

Prasugrel markedly inhibits ADP-, AA- and ristocetin-dependent platelet aggregation

Prasugrel loading significantly blunted various platelet-activating pathways compared with placebo as displayed in Figures 1 and 2. As expected, ADP-induced platelet aggregation decreased by 82% 2 h after prasugrel loading and stayed at that level for 24 h. Endotoxaemia did not affect VASP phosphorylation (Figure 1).

Prasugrel likewise significantly reduced AA- and ristocetin-induced platelet aggregation. Both platelet aggregation pathways were consistently suppressed by approximately 60% (AA) to 75% (ristocetin) as compared with baseline (both P < 0.0001 compared with placebo). LPS infusion had no impact on ADP- or AA-dependent platelet aggregation. In contrast, ristocetin-induced aggregation decreased by approximately 30% at 4 h (P = 0.001), which was followed by a 30% increase at 24 h (P = 0.02: Figure 2).
Prasugrel blunts various platelet activation pathways

A total of 16 subjects received two treatments consisting of 60 mg of prasugrel (solid squares) and placebo (open circles) followed by an intravenous bolus of endotoxin (2 ng/kg of body weight), four subjects received prasugrel (solid up-triangles) or placebo (open down-triangles) only (without LPS) and served as internal control. Results are means ± 95% CI or means only (control groups without LPS). The antiplatelet effect of prasugrel was measured by the MEA and the agonists AA (top left) and ADP (top right) and by the VASP assay (bottom left). Additionally, platelet counts are depicted (bottom right) during endotoxaemia. *P < 0.05 and **P < 0.0001 compared with placebo/LPS period. U, units.

Prasugrel has only a moderate effect on collagen and TRAP-dependent platelet aggregation

Collagen-induced platelet aggregation decreased by 27% as compared with baseline 2 h after prasugrel loading (Figure 2; P < 0.0001 compared with placebo). Interestingly, platelet inhibition diminished during endotoxaemia (−9% as compared with baseline 6 h after the LPS bolus; P < 0.05 compared with placebo). TRAP-dependent platelet aggregation decreased by 13% as compared with baseline 2 h after prasugrel loading (Figure 2; P < 0.05 compared with placebo) and reached nadir values 4 h after LPS infusion (−29% as compared with baseline). However, LPS also slightly decreased TRAP-induced aggregation by approximately 15% compared with baseline at 6 h, whereas collagen-induced aggregation slightly increased by 12% at 6 h.

Endotoxaemia attenuates the antiplatelet effects of prasugrel under high shear rates as measured with the PFA-100 system

Prasugrel loading markedly prolonged the PFA CT (>300 s) as measured with the new INNOVANCE® P2Y cartridge in all study subjects (Figure 3; P < 0.0001 compared with placebo). Of note, endotoxaemia did not negatively affect the sensitivity of INNOVANCE® P2Y cartridges, because CT values remained maximally prolonged. Prasugrel also inhibited shear-dependent platelet function as measured with the PFA-100 system: CADP-CT increased by 126% and CEPI-CT by 59%, compared with baseline 2 h after prasugrel loading (Figure 3; CADP-CT, P < 0.0001 compared with placebo; CEPI-CT, P < 0.05 compared with placebo). Thereafter, LPS infusion shortened CADP-CT and CEPI-CT and both reached nadir values 6 h after the LPS bolus (CADP-CT: −53% as compared with peak value; CEPI-CT: −61% as compared with peak value). CEPI-CT even fell to the very short CT values observed in the placebo period. CADP-CT and CEPI-CT remained stable in the control group without LPS.

As expected [8,32], vWF:Ag levels increased from 82% (95% CI, 70–93%) more than 3-fold to 270% (95% CI, 215–324%) 4 h after LPS treatment without any significant effect of prasugrel. vWF:Ag levels did not change in the control subjects without LPS.

DISCUSSION

The present clinical trial evaluated for the first time, in a randomized, double-blind, placebo-controlled fashion,
Prasugrel abrogates ristocetin-dependent platelet aggregation, but has only a mild effect on collagen and TRAP-dependent aggregation during endotoxaemia

A total of 16 subjects received two treatments consisting of 60 mg prasugrel (solid squares) and placebo (open circles) followed by an intravenous bolus of endotoxin (2 ng/kg of body weight), four subjects received prasugrel (solid up-triangles) or placebo (open down-triangles) only (without LPS) and served as internal control. Results are means ± 95% CI or means only (control groups without LPS). The antiplatelet effect of prasugrel was measured by the MEA and the following agonists: ristocetin (top; RISTO), collagen (middle; COL) and TRAP (bottom). ∗ P < 0.05 and ∗∗ P < 0.0001 compared with placebo period. U, units.

the effects of in vivo P2Y12 receptor blockade on various pathways of platelet aggregation in whole blood. Of interest, the P2Y12 antagonist prasugrel significantly blunted a broad spectrum of platelet aggregation pathways. Prasugrel markedly decreased ADP (−81%), AA (−60%) and ristocetin (−75%) induced platelet aggregation (Figures 1 and 2). In contrast, prasugrel only moderately inhibited collagen (−27%) and TRAP (−29%) induced platelet aggregation (Figure 2). However, the effects of prasugrel are partially reversed by vWF release as measured by CADP-CT in the PFA-100 system.

The inhibition of a broad range of platelet aggregation pathways after strong P2Y12 receptor blockade is supported by various preclinical studies. First, antagonism to platelet P2Y12 receptor can inhibit platelet activation and aggregation mediated by TXA2 (thromboxane A2) pathways, both by reducing platelet production of TXA2 and by inhibiting responses following TP (TXA2/prostaglandin H2) receptor activation [34–35]. Secondly, the P2Y12 receptor participates in collagen-induced aggregation, as shown by the reduced amplitude of aggregation and the prolongation of the lag phase from the addition of collagen to the onset of aggregation in knockout platelets [36]. In addition, the P2Y12 receptor plays an essential role in the platelet shape change induced by collagen when TXA2 formation is prevented [37]. Thirdly, the P2Y12 receptor plays a key role in the irreversible platelet aggregation through the thrombin receptor PAR1 [38,39]. Moreover, Armstrong et al. [40] recently demonstrated in vitro that strong P2Y12 receptor blockade with the prasugrel active metabolite R-138727 alone inhibited platelet aggregation in response to a variety of platelet agonists (AA, ADP, collagen, adrenaline and TRAP). As a consequence, aspirin may have little additional anti-platelet effect in healthy volunteers receiving prasugrel [41]. Consistent with our results, the effect of R-138727 on AA- and ADP-dependent platelet aggregation was much more pronounced than on collagen- or TRAP-dependent aggregation.
Our trial for the first time demonstrates that prasugrel substantially prolongs CEPI-CT and CADP-CT. This is in contrast with clopidogrel, which usually has only weak effects on CADP-CT [42,43], and indicates that stronger P2Y12 inhibition has a more pronounced effect on CADP-CT. In addition, the unequivocal prolongation of the P2Y-CT by prasugrel validates the INNOVANCE® P2Y cartridge, which was designed to overcome the sensitivity problems of CADP-CT for clopidogrel. The P2Y-CT was unaffected by the endotoxaemia-induced vWF release. In contrast, and as expected [8,11,33,44], LPS infusion markedly enhanced shear-dependent platelet function as measured by CEPI-CT or CADP-CT with the PFA-100 system, by releasing the acute phase protein vWF [8,32]. Of note, LPS infusion counteracted the effects of prasugrel on CADP-CT and CEPI-CT, which even declined to values below baseline during endotoxaemia, reaching placebo values (Figure 3). This is an interesting finding, which might have clinical implications, because patients with acute myocardial infarction complicated by cardiogenic shock and treated with stent placement often experience secondary infections or systemic inflammatory response syndrome with vWF release [45,46]. In addition, at least the resorption rate of the thienopyridine clopidogrel is markedly reduced in patients with myocardial infarction and haemodynamic instability due to reduced bowel perfusion [47]. As both CEPI-CT and CADP-CT have been found to predict the degree of myocardial necrosis in patients with myocardial infarction [12,48] as well as future cardiovascular events [13,14], these results may have broader clinical implications. Of interest, inflammatory biomarkers and ADP-induced platelet aggregation are significantly associated with CAD (coronary artery disease) patients under dual anti-platelet therapy [49]. Beyond that, high baseline CRP values and high on-treatment residual platelet aggregation have been independent predictors of adverse cardiac events in a large CAD-patient cohort after stent implantation [50].

Our trial, supported by a previous in vitro study [40], indicates that strong P2Y12 inhibition does not optimally block TRAP, collagen or shear-induced vWF-mediated aggregation. This is particularly true when stimuli such as collagen, vWF and high shear rates are combined in the PFA-100 system cartridges or in stenotic arteries when plaques are rupturing. Thus, one could expect synergistic effects from PAR1 antagonists, or vWF inhibitors such as ARC1779 or ALX0081, which are currently in clinical development [17,51].

LPS infusion had no major effect on platelet aggregation in response to ADP, AA or collagen, whereas TRAP-induced aggregation slightly decreased after LPS infusion. This is in good agreement with a subtle decrease in PAR1 receptors and TRAP-induced P-selectin expression after LPS [11]. However, ristocetin-induced aggregation decreased by approximately 30 % 4 h after LPS infusion. One may speculate that this could be due to removal of a responsive platelet subpopulation from the circulation during release of high-molecular-mass multimers of vWF [52], similar to what can be observed in vWD type 2b [31]. This concept could deserve further investigation with specific vWF inhibitors in the future. However, ristocetin-induced aggregation increased by 30% after 24 h, which corresponded to a partial loss of prasugrel’s effect on this pathway after 24 h (Figure 2).

A clear limitation of our trial is that our study findings are based on healthy volunteers and cannot easily be extrapolated to patients with systemic inflammation. For example, a platelet fall of approximately 15% was observed in our subjects during endotoxaemia, whereas patients with sepsis or even septic shock experience a much more pronounced platelet fall. The increased risk of bleeding may preclude the use of anti-platelet drugs in these patients. Moreover and as previously discussed [53–55], our inflammation model, although standardized and highly reproducible, is not a sepsis model. Similarly, the model is also not representative for bacteremia, where the situation may become even more complex, when Gram-positive bacteria may induce adhesion, platelet activation, or release of cytokines from platelets [56–58]. Thus, extrapolation to septic patients is not easily possible. Another possible limitation is that we did not use classical LTA (light transmission aggregometry) for determination of agonist-induced platelet aggregation. However, MEA and LTA show a good correlation in various trials [59] and MEA is less operator-dependent than LTA. Furthermore, the acute inflammation evoked by endotoxaemia cannot necessarily be extrapolated to chronic or more severe inflammatory conditions. In contrast, the study is strengthened by a solid study design (randomized-placebo controlled, two-way cross-over trial with two additional control groups) and use of different methods to measure P2Y12 blockade and platelet aggregation.

In conclusion, strong in vivo blockade of P2Y12 inhibits a broad spectrum of platelet aggregation pathways during acute systemic inflammation. In our model, there was no direct interaction between endotoxaemia and prasugrel. The observed interaction on the PFA-100 system values is probably due to vWF-release, which may, however, also weaken the effects of prasugrel under high-shear conditions in vivo.

AUTHOR CONTRIBUTION

Alexander Spiel contributed to the conception and design, acquisition, analysis and interpretation of data, and drafting of the paper. Ulla Derhaschnig contributed to the conception and design, acquisition of data, and revising the paper for important intellectual content.
Michael Schwameis contributed to the acquisition of data and revising the article for important intellectual content. Johann Bartko contributed to the acquisition of data and revising the article for important intellectual content. Jolanta Siller-Matula analysed and interpreted the data, and revised the article for important intellectual content. Bernd Jilma conceived, designed, analysed and interpreted the data, and revised the paper for important intellectual content. All authors finally approved the final version to be published.

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