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DIABETIC Medicine

RESEARCH: COMPLICATIONS

Protease-activated receptor-mediated platelet aggregation in patients with type 2 diabetes on potent $P2Y_{12}$ inhibitors

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Abstract

Background: Antiplatelet therapy is a cornerstone in the secondary prevention of ischemic events following percutaneous coronary intervention (PCI). The new $P2Y_{12}$ receptor inhibitors prasugrel and ticagrelor have been shown to improve patients' outcomes. Whether or not these drugs have equal efficacy in individuals with or without diabetes is disputed. Furthermore, platelets can be activated by thrombin, which is, at least in part, independent of $P2Y_{12}$-mediated platelet activation. Protease-activated receptor (PAR)-1 and −4 are thrombin receptors on human platelets. We sought to compare the in vitro efficacy of prasugrel ($n = 121$) and ticagrelor ($n = 99$) to inhibit PAR-mediated platelet aggregation in individuals with type 2 diabetes (prasugrel $n = 26$, ticagrelor $n = 29$).

Materials and Methods: We compared $P2Y_{12}$-, PAR-1- and PAR-4-mediated platelet aggregation as assessed by multiple electrode platelet aggregometry between prasugrel- and ticagrelor-treated patients without and with type 2 diabetes who underwent acute PCI.

Results: Overall, there were no differences of $P2Y_{12}$-, PAR-1- and PAR-4-mediated platelet aggregation between prasugrel- and ticagrelor-treated patients. However, both drugs inhibited $P2Y_{12}$-mediated platelet aggregation stronger, and thereby to a similar extent in patients with type 2 diabetes than in those without diabetes. There was no correlation between either $P2Y_{12}$-, or PAR-1- or PAR-4-mediated platelet aggregation and levels of HbA1c or the body mass index (BMI). However, we observed patients with high residual platelet reactivity in response to PAR-1 and PAR-4 stimulation in all cohorts.

Conclusion: Prasugrel and ticagrelor inhibit $P2Y_{12}$- and PAR-mediated platelet aggregation in individuals with diabetes to a similar extent, irrespective of HbA_{1c} levels and BMI.

KEYWORDS

acute coronary syndrome, antiplatelet therapy, diabetes, platelet thrombin receptors, prasugrel, ticagrelor

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1 | INTRODUCTION

Cardiovascular disease is a major cause of death and disability in patients with diabetes.[1] They frequently suffer from coronary artery disease often resulting in myocardial infarction (MI). Accordingly, many individuals with diabetes need to undergo percutaneous coronary intervention (PCI) with stent implantation. Secondary prevention following acute PCI comprises dual antiplatelet therapy (DAPT) with aspirin and one of the newer potent ADP $P2Y_{12}$ receptor blockers prasugrel or ticagrelor.[2]

Clinical studies indicate, however, that despite state-of-the art DAPT approximately 10% have additional ischemic events.[3–5] Whether prasugrel or ticagrelor are more advantageous following acute PCI is a matter of ongoing debate,[4,6] and in particular for patients with type 2 diabetes, this question cannot be definitively answered by clinical outcome data, so far. The ISAR-REACT 5 trial revealed no significant differences between the two drugs with respect to the 1-year outcome comprising MI, death, or ischemic stroke in patients with diabetes, but prasugrel to be the better choice over ticagrelor in the overall study population.[4] However, the open-label design of ISAR-REACT 5 is an inherent limitation of the trial.[6] Further analyses of this population confirmed the efficacy of ticagrelor to be comparable with that of prasugrel in patients with diabetes.[7]

A variety of conditions may, particularly in individuals with diabetes, lead to an impaired response to antiplatelet therapy. First, platelet turnover may be accelerated in diabetes, and therefore young platelets that are naive to inhibitory drugs may be more present in the circulation,[8] particularly if administered once (prasugrel) versus twice daily (ticagrelor). Second, the enteric resorption of any drug can be affected in diabetes.[9] Therefore, the required in vivo concentration of the drug to achieve platelet inhibition may not be reached. Thereby, resorption may differ from one drug to the next. Third, prasugrel but not ticagrelor needs to be metabolized to become a potent platelet inhibitor but metabolism may be impaired in diabetes.[10] Fourth, alterations of the platelet membrane due to hyperglycaemia and failure of insulin to inhibit platelet signalling may be responsible for an increased reactivity to ADP.[11] Fifth, other clinical conditions known to be associated with high on-treatment residual platelet reactivity to ADP (HRPR ADP), which are frequent in individuals with diabetes might play a role, such as kidney failure,[12] high body mass index (BMI),[13] inflammation[14] and drug–drug interactions.[10] These conditions may affect the antiplatelet potency of one drug more than that of the other one.

Adverse ischemic outcomes after PCI may also be attributable to intact platelet aggregation via the human thrombin receptors protease-activated receptor (PAR)-1 and PAR-4,[15–17] overcoming $P2Y_{12}$ inhibition. Thrombin is a very strong endogenous platelet agonist[15] and its ongoing generation has been particularly recognized in diabetes and/or obese patients.[18,19] Thrombin-mediated platelet activation is not specifically targeted by current state-of-the-art DAPT with aspirin and a potent $P2Y_{12}$ antagonist following MCI, and may therefore reflect $P2Y_{12}$-independent platelet activation. Moreover, potential differences between prasugrel and ticagrelor in the degree of $P2Y_{12}$ inhibition would be amplified due to the synergism between ADP and thrombin for platelet activation.

We, therefore, evaluated platelet response to in vitro PAR-1 and PAR-4 stimulation, the two important thrombin receptors on human platelets, in patients with diabetes receiving either prasugrel or ticagrelor after PCI. Differences between the two drugs may affect the treatment of individuals with diabetes following acute PCI.

Novelty statement

- Prasugrel and ticagrelor effectively prevent secondary cardiovascular events, by inhibiting the $P2Y_{12}$ receptor. However, platelets are also activated by thrombin via protease-activated receptor (PAR)-1 and PAR-4, activation pathways that may be responsible for recurrent ischemic events, particularly in patients with diabetes.
- Inhibition of the $P2Y_{12}$ receptor by prasugrel and ticagrelor is stronger in individuals with type 2 diabetes than in those without diabetes.
- PAR-1- and PAR-4-mediated platelet aggregation is inhibited by both drugs to a similar extent.
- Prasugrel and ticagrelor provide equal platelet inhibition in all patient cohorts, irrespective of HbA1c levels and BMI.

2 | PATIENTS

Patients were enrolled consecutively at the Department of Internal Medicine II, Division of Cardiology, at the Medical University of Vienna. No patient declined participation in this observational study. The study population included 220 consecutive patients with MI, receiving DAPT with aspirin (loading dose of 250 mg, thereafter 100 mg once daily), and either prasugrel (loading dose of 60 mg, thereafter 10 mg once daily, $n = 121$), or ticagrelor (loading dose of 180 mg, thereafter 90 mg twice daily, $n = 99$), before acute PCI and stenting. There were 165

patients without diabetes and 55 patients with type 2 diabetes. The diagnosis of type 2 diabetes was based on the patients' history, an HbA_{1c} value >6.5% and regular antidiabetic therapy. The selection of the respective $P2Y_{12}$ inhibitor was at the discretion of the attending physician. All individuals were Caucasians from the Viennese urban area. All patients gave their written informed consent for participation. We excluded patients who had any major surgery during the last week before enrolment, a treatment with vitamin K antagonists (phenprocoumon, acenocoumarol, warfarin), rivaroxaban, apixaban, dabigatran, or edoxaban. Moreover, patients were excluded if they were taking nonsteroidal anti-inflammatory drugs, ticlopidine, or dipyridamole. We also excluded patients with known bleeding disorders, severe hepatic failure, known qualitative defects in platelet function, a history of heparin-induced thrombocytopenia or malignant myeloproliferative disorders. Patients were not eligible if they had a platelet count <100,000/μl or >450,000/μl, or a haematocrit <30%.

The study was approved by the Ethics Committee of the Medical University of Vienna in accordance with the declaration of Helsinki and its later amendments.

3 | MATERIALS AND METHODS

All blood samples, including those for routine laboratory evaluations were obtained median 72 h (range 66–74 h) after PCI, when both prasugrel and ticagrelor were at, or approached the steady state.[20,21] Blood sampling was performed in the morning after an overnight fast at the same time point in all patients, irrespective of the prescribed $P2Y_{12}$ inhibitor and the presence or absence of diabetes.

A butterfly needle (21-gauge, 0.8×19 mm; Greiner Bio-One) was inserted by aseptic venepuncture into an antecubital vein. All blood samples were collected by the same physician. Periprocedural platelet activation was avoided by discharging the first 3 ml of blood. Blood samples were collected in hirudin-coated tubes (Roche Diagnostics), which then were gently inverted immediately. All samples were investigated by whole blood impedance aggregometry (multiple electrode aggregometry, MEA), as previously described.[22] One Multiplate test cell contains two independent sensor units and one unit consists of 2 silver-coated highly conductive copper wires with a length of 3.2 mm. After dilution (1:2 with 0.9% NaCl solution) of hirudin-anticoagulated whole blood and stirring in the test cuvettes for 3 min at 37°C, the agonists adenosine diphosphate (ADP, $P2Y_{12}$ agonist, 6.4 μM), L-seryl-L-phenylalanyl-L-leucyl-L-leucyl-L-arginyl-L-asparagine (SFLLRN, PAR-1 agonist, 32 μM) or alanyl-L-tyrosyl-L-prolyl-glycyl-L-lysyl-L-phenylalanine (AYPGKF, PAR-4 agonist, 645 μM; all from Roche Diagnostics), were added and aggregation was continuously recorded for 6 min. The respective concentrations of agonists have been established in our laboratory by titration experiments resulting in less than maximal platelet aggregation (60%–75% of maximal aggregation) in healthy individuals ($n = 30$).[22] The adhesion of activated platelets to the electrodes led to an increase of impedance, which was detected for each sensor unit separately and transformed to aggregation units (AU) that were plotted against time. The AU at 6 min were used for calculations. The thresholds for HRPR ADP, HRPR SFLLRN and HRPR AYPGKF were ≥47, >71 and >54 AU, respectively.[22,23]

3.1 | Statistical analysis

A sample size calculation was based on the observed mean ± SD of AYPGKF-inducible platelet aggregation by MEA (64 ± 19 AU) in a population of 20 patients with type 2 diabetes on DAPT with aspirin and prasugrel 72 hours after acute angioplasty and stenting. We calculated that we needed to include 52 patients (26 per group) to be able to detect a 25% relative difference of AYPGKF-inducible platelet aggregation by MEA between prasugrel- and ticagrelor-treated patients with type 2 diabetes with a power of 85% (using a two-sided alpha level of 0.05).

Continuous data are shown as median and interquartile range whereas categorical data are depicted as number and percentage. The non-parametric Mann–Whitney U-test was used to assess differences between continuous variables in patients on prasugrel or ticagrelor. Furthermore, data were analysed by two-way analysis of variance (ANOVA) on rank-transformed values, including treatment groups with prasugrel and ticagrelor, and the presence and absence of diabetes into the model. Additionally, an interaction term was tested to evaluate potential differences in the diabetes effect depending on the assigned drug. The chi-squared test was used to calculate differences between categorical variables. Spearman correlation was used to assess correlations. We used the Statistical Package for Social Sciences (SPSS version 24.0; SPSS) to conduct all statistical analyses. Two-sided $p < 0.05$ were considered statistically significant.

4 | RESULTS

Clinical, laboratory and procedural characteristics of patients with type 2 diabetes and patients without diabetes receiving prasugrel or ticagrelor are shown in Table 1. The study cohort comprised 121 patients on prasugrel and 99 patients on ticagrelor. In the prasugrel and the ticagrelor group 26 and 29 patients, respectively, had

TABLE 1 Characteristics of patients with type 2 diabetes and patients without diabetes receiving prasugrel or ticagrelor

	Prasugrel ($n = 121$)			Ticagrelor ($n = 99$)			*p*-value prasugrel vs ticagrelor
	Diabetes ($n = 26$)	No diabetes ($n = 95$)	*p*-value	Diabetes ($n = 29$)	No diabetes ($n = 70$)	*p*-value	
Demographics							
Age (years)	60 (55–66)	55 (46–62)	0.03	63 (56–74)	56 (50–68)	0.05	0.38
Men, *n* (%)	20 (77)	77 (81)	0.64	22 (76)	47 (67)	0.01	0.07
BMI (kg/m^2)	28 (26–30)	28 (25–31)	0.51	28 (26–33)	27 (24–30)	0.09	0.69
Medical history, *n* (%)							
Previous MI	4 (15)	14 (15)	0.93	9 (31)	7 (10)	0.01	0.79
Hypertension	19 (73)	59 (61)	0.30	29 (100)	60 (86)	0.12	<0.001
Active smoking	16 (62)	56 (59)	0.81	11 (38)	33 (47)	0.53	0.03
Stent implantation	26 (100)	95(100)	1.00	29 (100)	70 (100)	1.00	1.00
Number of stents/ patient	1 (1–2)	1 (1–2)	1.00	1 (1–2)	1 (1–2)	1.00	1.00
Laboratory data							
HbA_{1c} (mmol/mol)	54 (43–69)	37 (34–39)	<0.001	51 (45–57)	37 (33–40)	<0.001	0.32
HbA_{1c} (%)	7.1 (6.05–8.5)	5.5 (5.3–5.7)	<0.001	6.8 (6.3–7.4)	5.50 (5.2–5.8)	<0.001	0.32
Serum creatinine (mg/dl)	0.92 (0.80–1.09)	0.87 (0.76–1.00)	0.30	1.06 (0.94–1.24)	0.95 (0.81–1.16)	0.08	<0.001
Platelet count (G/L)	198 (156–237)	199 (166–228)	0.1	182 (143–221)	192 (170–256)	0.26	0.67
High sensitivity C-reactive protein (mg/dl)	2.65 (0.90–5.59)	1.26 (0.73–2.60)	0.052	1.42 (0.48–4.58)	1.24 (0.36–2.33)	0.42	0.21
Hemoglobin (g/dl)	13.90 (13.25–14.95)	13.95 (13.10–14.78)	0.91	13.55 (12.33–14.68)	14.10 (12.90–14.70)	0.45	0.39
White blood cell count (G/L)	10.31 (8.49–11.96)	8.57 (7.69–10.06)	0.14	8.70 (6.98–11.35)	9.39 (6.71–10.32)	0.90	0.63
Medication, *n* (%)							
Statins	26 (100)	92 (97)	0.90	29 (100)	58 (83)	0.66	0.01
Beta blockers	26 (100)	89 (94)	0.88	29 (100)	57 (81)	0.49	0.03
ACE inhibitors	23 (88)	77 (81)	0.38	21 (72)	47 (67)	0.60	0.02
Calcium channel blockers	3 (12)	7 (7)	0.49	4 (14)	6 (9)	0.45	0.60
Angiotensin receptor blockers	4 (15)	13 (14)	0.83	8 (28)	19 (27)	0.96	0.02
Diabetes therapy, *n* (%)							
GLP-1-receptor-agonists	0 (0)	0 (0)		1 (3)	0 (0)		0.98
Gliptins	5 (19)	0 (0)		8 (28)	0 (0)		0.45
Sulfonylureas	3 (12)	0 (0)		4 (14)	0 (0)		0.80
Metformin	16 (62)	0 (0)		11 (38)	0 (0)		0.08
Glitazones	1 (4)	0 (0)		1 (3)	0 (0)		0.93
SGLT-2 inhibitors	4 (15)	0 (0)		3 (10)	0 (0)		0.31

type 2 diabetes. Patients with diabetes treated with either prasugrel or ticagrelor were younger than individuals without diabetes, indicating their earlier onset of atherosclerosis. Patients on ticagrelor had only mildly elevated, but significantly higher serum creatinine levels (Table 1), possibly attributable to the treatment with ticagrelor,[24] which had been commenced before PCI. As expected, patients with diabetes in both, prasugrel and

ticagrelor groups, had significantly higher HbA_{1c} levels (Table 1).

4.1 | Residual platelet aggregation in response to ADP/ SFLLRN/AYPGKF

We first evaluated residual platelet response to ADP in prasugrel- and ticagrelor-treated patients. Patients on prasugrel responded similarly to platelet activation by ADP as patients on ticagrelor (Figure 1a). Of note, we observed significantly lower residual platelet aggregation in response to ADP in individuals with type 2 diabetes as compared to those without diabetes (Figure 1b). This association was not dependent on the assigned drug (p for interaction = 0.335). Of note, two patients without diabetes had HRPR ADP as they had AU ≥47, the cut-off for adequate platelet inhibition by ADP $P2Y_{12}$ receptor antagonists. Their platelet responses to SFLLRN were 92 and 106 AU, and to AYPGKF 62 and 100 AU, respectively.

Patients on prasugrel responded similarly to platelet activation by SFLLRN as patients on ticagrelor (Figure 2a). Furthermore, there was no significant difference regarding the response to SFLLRN between individuals with type 2 diabetes compared to those without diabetes (Figure 2b). Patients on prasugrel responded similarly to platelet activation by AYPGKF as patients on ticagrelor (Figure 3a). Moreover, there was no significant difference between patients with type 2 diabetes compared with those without diabetes (Figure 3b). However, there was a trend towards stronger inhibition in individuals with type 2 diabetes treated with ticagrelor (p value for interaction 0.058). In the prasugrel group 119 patients had on-treatment residual platelet reactivity below the cut-off for HRPR ADP. Of these, 51 patients (43%) had HRPR SFLLRN and 71 (60%) had HRPR AYPGKF. In the population with diabetes, we identified 12 patients (46%) with HRPR SFLLRN and 16 patients (62%) with HRPR AYPGKF.

All ticagrelor-treated patients had on-treatment residual platelet reactivity below the cut-off for HRPR ADP. Of

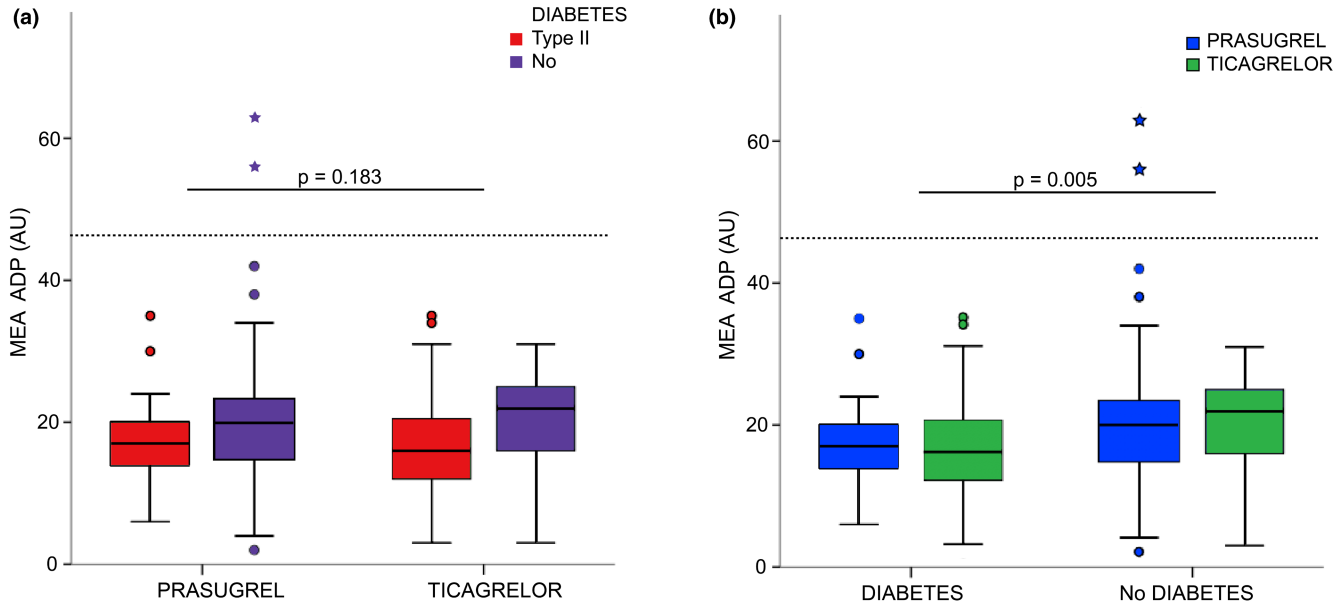

FIGURE 1 (a) Platelet response to adenosine diphosphate (ADP) in patients on prasugrel compared to patients on ticagrelor. Patients received either prasugrel (n = 121, 26 with type 2 diabetes) or ticagrelor (n = 99, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes length. Outliers are shown by full circles and extremes by asterisk. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (≥47 AU). Data were analysed by two-way ANOVA on rank-transformed values, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The p-value results from the two-way ANOVA model comparing treatment groups. AU, aggregation units; MEA, multiple electrode aggregometry. (b) Platelet response to adenosine diphosphate (ADP) in patients with type 2 diabetes compared to patients without diabetes. Patients received either prasugrel (n = 121, 26 with type 2 diabetes) or ticagrelor (n = 99, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes' length. Outliers are shown by full circles and extremes by asterisk. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (≥47 AU). Data were analysed by two-way ANOVA on rank-transformed values, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The p-value results from the two-way ANOVA model comparing patients with diabetes to patients without diabetes. AU, aggregation units; MEA, multiple electrode aggregometry

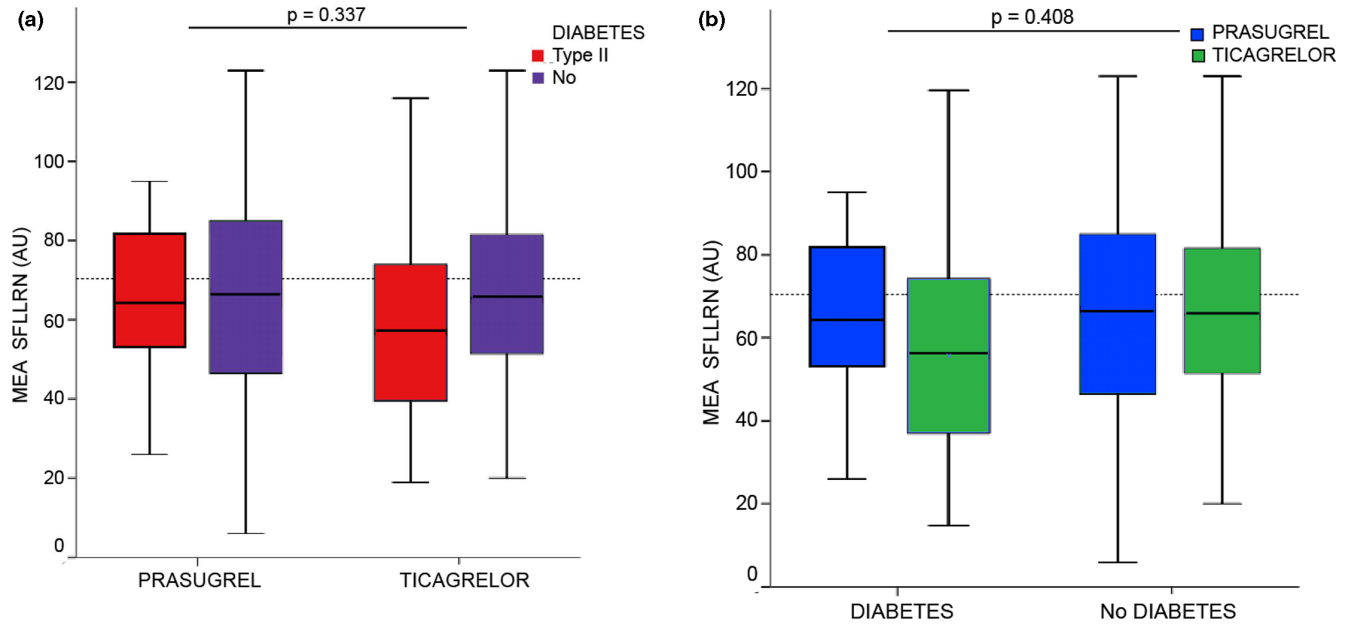

FIGURE 2 (a) Platelet response to SFLLRN in patients on prasugrel compared to patients on ticagrelor. Patients received either prasugrel ($n = 121$, 26 with type 2 diabetes) or ticagrelor ($n = 99$, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes length. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (>71 AU). Data were analysed by two-way ANOVA on ranks, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The p-value indicates significance between treatment groups by two-way ANOVA analysis. AU, aggregation units; MEA, multiple electrode aggregometry. (b) Platelet response to SFLLRN in patients with type 2 diabetes compared to patients without diabetes. Patients received either prasugrel ($n = 121$, 26 with type 2 diabetes) or ticagrelor ($n = 99$, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes length. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (>71 AU). Data were analysed by two-way ANOVA on ranks, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The p-value indicates significance between groups with type 2 diabetes or without diabetes by two-way ANOVA analysis. AU, aggregation units; MEA, multiple electrode aggregometry

these patients, 30 (30%) had HRPR SFFLRN and 55 (56%) had HRPR AYPGKF. In the population with type 2 diabetes, seven patients (24%) had HRPR SFLLRN and 11 patients (38%) had HRPR AYPGKF. In individuals with type 2 diabetes, HRPR in response to SFLLRN and AYPGKF did not significantly differ between those on prasugrel versus those on ticagrelor ($p = 0.400$, $p = 0.485$, respectively). In addition, in the cohort comprising patients without diabetes there were no significant differences between prasugrel and ticagrelor with respect to the two different agonists ($p = 0.098$ and $p = 0.292$, for SFLLRN and AYPGKF, respectively).

As $P2Y_{12}$ inhibition also affects the response to PAR stimulation we subsequently assessed the correlations between on-treatment residual platelet reactivity in response to ADP and in response to both PAR agonists, SFLLRN and AYPGKF. Patients with type 2 diabetes were evaluated separately from patients without diabetes. We observed a similar correlation between the responses to ADP and SFLLRN in patients with and without type 2 diabetes on prasugrel and ticagrelor (Table 2). There was also a significant correlation between the response to ADP and AYPGKF in individuals with type 2 diabetes on prasugrel and ticagrelor, as well as in the cohort without diabetes (Table 2).

The two platelet thrombin receptors PAR-1 and PAR-4 play a significant mutual role in platelet activation. Therefore, it was of interest to determine if the residual responsiveness to the specific PAR agonists SFLLRN and AYPGKF correlates similarly in patients with type 2 diabetes compared with patients without diabetes treated with prasugrel or ticagrelor. We observed significant correlations between the responses to SFLLRN and AYPGKF in the cohort with type 2 diabetes (Table 2) and in the cohort of patients without diabetes (Table 2).

4.2 | Residual platelet aggregation in response to ADP/SFLLRN/AYPGKF and correlation with HbA_{1c}

Metabolic control may influence the responsiveness to DAPT. We therefore correlated levels of HbA_{1c}, as a measure

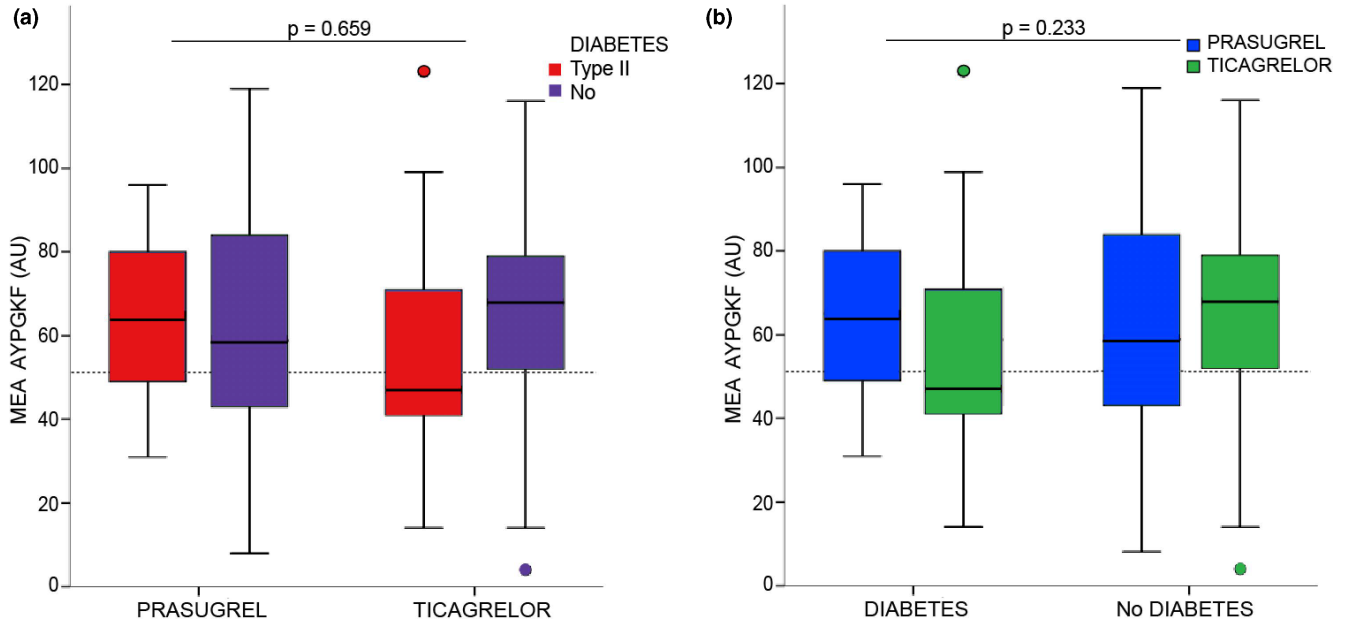

FIGURE 3 (a) Platelet response to AYPGKF in patients on prasugrel compared to patients on ticagrelor. Patients received either prasugrel ($n = 121$, 26 with type 2 diabetes) or ticagrelor ($n = 99$, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes length. Outliers are shown by full circles. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (>54 AU). Data were analysed by two-way ANOVA on ranks, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The *p*-value indicates significance between treatment groups by two-way ANOVA analysis. AU, aggregation units; MEA, multiple electrode aggregometry. (b) Platelet response to AYPGKF in patients with type 2 diabetes compared to patients without diabetes. Patients received either prasugrel ($n = 121$, 26 with type 2 diabetes) or ticagrelor ($n = 99$, 29 with type 2 diabetes). The boundaries of the box show the lower and upper quartile of data. The line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the boxes' length. Outliers are shown by full circles. The dotted line indicates the threshold for high on-treatment residual platelet reactivity (>54 AU). Data were analysed by two-way ANOVA on ranks, including prasugrel, ticagrelor, and the presence or absence of type 2 diabetes into the model. The *p*-value indicates significance between groups with type 2 diabetes or without diabetes by two-way ANOVA analysis. AU, aggregation units; MEA, multiple electrode aggregometry

of long-term blood glucose control, with on-treatment residual platelet aggregation in response to ADP. There was no significant correlation between levels of HbA_{1c} and the response to ADP in the study population (Table 2).

Based on the assumption that impaired diabetic control influences particularly thrombin-inducible platelet activation, we also assessed the correlation of HbA_{1c} levels with the platelet response to the PAR-1 and -4 agonists SFLLRN and AYPGKF, respectively. There were no significant correlations between levels of HbA_{1c} and the response to SFLLRN or AYPGKF in the study population (Table 2).

4.3 | Residual platelet aggregation in response to ADP/SFLLRN/AYPGKF and correlation with BMI

As high BMI levels may impair the response to treatment with prasugrel or ticagrelor we assessed the correlation between BMI and the residual response to ADP. There was no significant correlation between levels of BMI and the response to ADP (Table 2). High levels of BMI have been associated with increased thrombin generation and PAR-1 mediated platelet aggregation.[14] We, therefore, anticipated a correlation between BMI and the responsiveness to the PAR-1 and PAR-4 agonists. However, there was no significant correlation between BMI and the response to SFLLRN, or the response to AYPGKF in the study population (Table 2).

5 | DISCUSSION

This study investigated if prasugrel and ticagrelor are equally potent inhibitors of PAR-1- and PAR-4-mediated platelet aggregation in individuals with type 2 diabetes. Our data show no significant differences between platelet PAR-1 and PAR-4 response in patients on prasugrel as

TABLE 2 Correlations of platelet responses to agonists with HbA_{1c} levels and BMI in patients with and without type 2 diabetes

Patients on prasugrel and ticagrelor: Correlation between	*r*-value	*p*-value
Responses to ADP and SFLLRN in individuals with type 2 diabetes	0.55	<0.001
Responses to ADP and SFLLRN in individuals without type 2 diabetes	0.56	<0.001
Responses to ADP and AYPGKF in individuals with type 2 diabetes	0.27	0.048
Responses to ADP and AYPGKF in individuals without type 2 diabetes	0.53	<0.001
Responses to SFLLRN and AYPGKF in individuals with type 2 diabetes	0.71	<0.001
Responses to SFLLRN and AYPGKF in individuals without type 2 diabetes	0.56	<0.001
HbA_{1c} and the response to ADP	0.07	>0.05
HbA_{1c} and the response to SFLLRN	0.09	>0.05
HbA_{1c} and the response to AYPGKF	0.07	>0.05
BMI and the response to ADP	−0.02	>0.05
BMI and the response to SFLLRN	0.06	>0.05
BMI and the response to AYPGKF	0.05	>0.05

Abbreviations: ADP, adenosine diphosphate; AYPGKF, alanyl-L-tyrosyl-L-prolyl-glycyl-L-lysyl-L-phenylalanine; BMI body mass index; SFLLRN, L-seryl-L-phenylalanyl-L-leucyl-L-leucyl-L-arginyl-L-asparagine.

compared to patients on ticagrelor. Accordingly, platelet aggregation in response to PAR-1 and PAR-4 stimulation was inhibited by both drugs to a similar extent. Moreover, the response to the agonists did not correlate with HbA_{1c} levels or BMI.

Prasugrel and ticagrelor inhibited platelet response to ADP to a similar extent, i.e. all patients on ticagrelor and all but two patients on prasugrel exhibited a residual platelet response below the internationally-agreed threshold of 47 AU for HRPR ADP. This threshold has been established based on clinical data from patients on clopidogrel therapy,[23] and may therefore need to be adjusted for the newer drugs.

In a previous report, prasugrel was found to inhibit platelet aggregation stronger than ticagrelor.[25] These findings were similar if samples were analysed median 11.8 or 38.5 h after loading with the respective $P2Y_{12}$ inhibitor.[25] We, however, did not see such an effect in our cohort, possibly due to the later time point of blood sampling, namely 72 h after PCI and stent implantation in our study. Of note, at this later time point patients had already received either prasugrel or ticagrelor for three consecutive days, possibly representing a better steady-state after the intervention.[20,21]

Unexpectedly, our data show that platelets from patients with type 2 diabetes are more susceptible to $P2Y_{12}$ inhibition by prasugrel and ticagrelor than patients without diabetes. Platelet aggregation however, is only one aspect of platelet function, and other platelet contributions to atherosclerosis need consideration in this inflammatory process.[26] As an example, ticagrelor exerts a stronger inhibitory effect on toll-like receptor-1/2 than prasugrel.[27] As diabetes often is associated with a status of increased inflammation and thus cytokine storms with up-regulated toll-like receptors, it may be speculated that prasugrel and ticagrelor, "calm" the innate immune system in diabetes to a different extent. Accordingly, Jeong et al. found a significant reduction of inflammatory markers in the ticagrelor group compared with prasugrel treated individuals.[28]

It has been shown that patients with diabetes have a higher thrombin generation potential than non-diabetic patients.[18] Further, it is possible that ongoing thrombin generation is a main reason for recurrent events despite potent inhibition of ADP- inducible platelet activation. By inhibiting $P2Y_{12}$, prasugrel and/or ticagrelor could alter the contribution of ADP to platelet phosphatidylserine exposure and thrombin generation at the platelet surface.[29] However, platelets, which have been successfully inhibited for ADP-inducible activation, may still be responsive to platelet activation via PAR.

We therefore assumed that particularly platelets from patients with type 2 diabetes are poised for activation by thrombin, and the exogenous addition of the respective platelet agonists for PAR-1 and PAR-4 will reveal higher aggregation values in individuals with diabetes as compared to those without diabetes. Alternatively, increased thrombin generation may lead to increased thrombin receptor response already in vivo and subsequent receptor downregulation would be followed by impaired responsiveness to in vitro added agonists. However, in the overall cohort, we saw no difference in the response to the PAR-directed agonists SFLLRN or AYPGKF in prasugrel- versus ticagrelor-treated patients. Further analyses also revealed no significant differences between patients with type 2 diabetes and those without diabetes with the two platelet inhibitors indicating their equal potency to suppress platelet activation via both PAR receptors. In our in vitro investigations, we observed a significant correlation between platelet response to ADP and the platelet response to both PAR agonists. This correlation was seen in patients with and without diabetes. The significant correlations between the residual response to ADP and SFLLRN or AYPGKF suggest that a large proportion of the variation in platelet response to the thrombin receptor agonists is due to variation in platelet response to ADP, as pathways of platelet activation are not independent of each other. However, among the patients who adequately responded to $P2Y_{12}$ inhibition by prasugrel or ticagrelor

we identified a number of individuals who still responded to activation by either SFLLRN or AYPGKF or both. The latter ones were seen equally frequent in the cohort comprising patients with type 2 diabetes as in patients without diabetes. We therefore conclude that both drugs are potent inhibitors of ADP-inducible platelet aggregation, but less potent inhibitors of PAR-mediated platelet aggregation, in patients with and without type 2 diabetes.

Metabolic control in individuals with diabetes may improve their response to antiplatelet therapy. Clinically, ticagrelor was shown to reduce the primary endpoint, all-cause mortality, and stent thrombosis in patients with levels of HbA_{1c} above the median.[30] High BMI levels have been associated with high thrombin generation potential.[19] We followed the hypothesis, that ongoing thrombin generation is responsible for increased platelet responsiveness to PAR-1- and PAR-4-mediated platelet activation. Thereby, we investigated, if platelet responsiveness to PAR-1 and PAR-4 stimulation is correlated with levels of HbA_{1c}, as an indicator of long-term metabolic control, and with BMI, a rough overall indicator of impaired metabolism, which can be associated with inflammation.[19] The data from our study indicate that neither HbA_{1c} levels nor BMI are associated with the response to platelet activation by the two PAR agonists. Apparently, the potency of the newer inhibitors prasugrel and ticagrelor can overcome the unfavourable conditions, like poor control of diabetes or high BMI, that have been associated with reduced platelet inhibition in the past.

5.1 | Study limitations

Due to the small number of patients with type 2 diabetes, our study should be considered hypothesis-generating only, and our findings need to be confirmed in larger populations. Furthermore, we used only an aggregation assay in this study. Platelet aggregation however, is only one aspect of platelet function, and further platelet features can be of importance given the fact that atherosclerosis is an inflammatory disease.[26]

Each patient sample was analysed only once. The laboratory staff was blinded to the origin of the samples. Samples were only obtained at a single time point, namely 72 h after PCI. We cannot rule out that results would be slightly different, if samples were analysed at other time points.

We cannot rule out that the cohort comprising patients without diabetes included a few patients with pre-diabetes. However, we were interested to investigate differences between overt diabetes and non-diabetes.

Our study was not designed to assess clinical outcomes. The aim of the study was the evaluation of the in vitro response to PAR agonists despite potent suppression of ADP-inducible platelet activation in individuals with type 2 diabetes. According to our data, a considerable number of both, patients with and without diabetes, show high residual platelet activation via PAR-1 and PAR-4 in vitro, without significant differences between prasugrel- and ticagrelor-treated patients. In patients with diabetes, it would be interesting to compare thrombin generation in prasugrel- and ticagrelor- treated patients, in particular since impaired glucose metabolism is associated with increased thrombin generation potential in patients undergoing angioplasty and stenting.[18] However, we did not measure thrombin generation potential in this study. Whether or not individuals, who still respond to PAR-mediated platelet aggregation, can benefit from inhibition of thrombin-mediated platelet activation, e.g. with the PAR-1 inhibitor vorapaxar[31] or the thrombin inhibitor dabigatran[32] needs to be evaluated in clinical trials. However, a therapeutic regimen aimed at PAR inhibition may be considered only for very high-risk patients with defined residual response to PAR-mediated platelet activation.

6 | CONCLUSION

To the best of our knowledge, this is the first study addressing PAR-1- and PAR-4-mediated in vitro platelet aggregation in patients with type 2 diabetes on DAPT with either prasugrel or ticagrelor. The results obtained with the two $P2Y_{12}$ blockers were similar, without significant differences of PAR-1- and PAR-4-mediated platelet aggregation between patients without and with type 2 diabetes. Moreover, levels of metabolic control, as estimated by HbA_{1c} and BMI showed no correlation with residual platelet response to ADP, or the PAR-1 and PAR-4 specific agonists.

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CONFLICT OF INTEREST

All authors declare that there are no relationships or activities that might bias, or be perceived to bias, their work.

CONSENT FOR PUBLICATION

All patients gave their written informed consent for participation.

ETHICS APPROVAL

The study was approved by the Ethics Committee of the Medical University of Vienna in accordance with the declaration of Helsinki and its later amendments.

DATA AVAILABILITY STATEMENT

Raw data generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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