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Full Length Article

Antiplatelet effect of aspirin during 24 h in patients with type 2 diabetes without cardiovascular disease☆

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ABSTRACT

Introduction: The antiplatelet effect of low-dose aspirin in patients with type 2 diabetes (T2DM) without cardiovascular disease (CVD) has not been thoroughly explored. We investigated if platelet aggregation increased during the standard 24-hour aspirin dosing interval in patients with T2DM compared to non-diabetic controls. Furthermore, we evaluated baseline platelet aggregation, the acute effects of aspirin on platelet aggregation and platelet turnover.

Materials and methods: We included 21 patients with T2DM and 21 age and sex-matched controls. Platelet aggregation was measured by impedance aggregometry (Multiplate® Analyzer) and markers of platelet turnover by flow cytometry (Sysmex® XE-5000). Blood samples were obtained at baseline and 1 h after administration of 75 mg of aspirin. Participants were then treated for 6 days with once-daily aspirin, and blood sampling was repeated 1 h and 24 h after aspirin intake.

Results: After 6 days of treatment, platelet aggregation levels increased during the 24-hour aspirin dosing interval in both patients and controls ($p < 0.001$) with no difference between patients and controls. At baseline, patients with diabetes had increased platelet aggregation compared to controls ($p = 0.03$). Platelet aggregation was reduced after the first dose of aspirin and significantly further reduced after six days of treatment ($p < 0.001$). Patients with T2DM had numerically higher immature platelet count compared to controls ($p = 0.09$), indicating an increased platelet turnover.

Conclusion: Patients with T2DM without a history of CVD and controls had increased platelet aggregation at the end of the standard 24-hour dosing interval of aspirin. Further, aspirin-naïve T2DM patients had increased platelet aggregation compared to controls.

1. Introduction

Diabetes mellitus confers a substantial excess risk of cardiovascular events compared to individuals without diabetes [1,2]. Aspirin inhibits platelet aggregation, and treatment with once-daily low-dose aspirin is a cornerstone in secondary prevention of cardiovascular disease (CVD) in patients with type 2 diabetes mellitus (T2DM) [3]. However, the role of aspirin in primary prevention of CVD remains controversial [4–6].

Patients with T2DM have accelerated platelet turnover [7,8], and it has been suggested that this may lead to a reduced antiplatelet effect of aspirin [9,10]. In addition, platelets in patients with T2DM are characterized by increased adhesion, activation and aggregation [11,12].

The acetylation of cyclooxygenase-1 (COX-1) by aspirin is irreversible and, since platelets cannot synthesize new COX-1, inhibition of thromboxane A_2 synthesis lasts for the lifespan of the platelet [13]. However, aspirin has a short plasma half-life of just 15–20 min and, thus, an increased platelet turnover may leave a subgroup of platelets uninhibited by aspirin when aspirin is only ingested once daily.

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Furthermore, newly produced platelets have a higher haemostatic potential, and therefore an increased number of immature platelets may reduce the effect of aspirin [14].

It has recently been demonstrated that platelet inhibition is reduced toward the end of the usual 24-hour dosing interval of aspirin in patients with T2DM and CVD [15]. Whether the effect of aspirin is declining during the dosing interval also in T2DM patients without established CVD is currently unknown. Moreover, the acute effect of low-dose aspirin in aspirin naïve diabetic subjects remains sparsely elucidated.

The first aim of this study was to assess platelet aggregation during the standard 24-hour dosing interval of aspirin in patients with T2DM without a history of CVD compared to age- and sex-matched non-diabetic controls.

The second aim was to assess platelet aggregation at baseline before aspirin treatment and 1 h after ingestion of the first dose of aspirin in order to investigate the acute effect of aspirin in the two groups compared to the effect after 6 days of treatment. The third aim was to study platelet turnover in both groups.

Our primary hypothesis was that platelet aggregation increased at the end of the 24-hour dosing interval in patients with T2DM without a history of CVD.

2. Materials and methods

2.1. Study population

Between May and December 2016, a total of 21 patients with T2DM and 21 age- and sex-matched controls were enrolled. Patients were recruited from the outpatient clinic at the Department of Endocrinology, Aarhus University Hospital, Denmark, and were eligible for participation if they were ≥ 18 years and had a diagnosis of T2DM. Controls were identified from an existing study cohort [16]. Diabetes was excluded by an oral glucose tolerance test and blood haemoglobin A_{1c} (HbA_{1c}) < 48 mmol/mol.

Exclusion criteria for both patients and controls were a previous history of CVD (defined as any record of cardio- or cerebrovascular events, a history of cardiovascular disease necessitating medical treatment, or cardiac arrhythmia), treatment with any anticoagulant or antiplatelet drug including aspirin, present cancer, acute or chronic infectious disease, renal disease, present gastrointestinal bleeding, pregnancy, platelet disorders and/or bleeding disorders, intake of non-steroidal anti-inflammatory drugs within 14 days of study participation, or platelet count < 120×10^9/L.

The study was carried out in accordance with the International Conference on Harmonization Good Clinical Practice guidelines (EudraCT 2016-000515-32) and complied with the Declaration of Helsinki. The Central Denmark Region Committees on Health Research Ethics (1-10-72-153-15), the Danish Data Protection Agency (1-16-02-109-16) and the Danish Medicines Agency (2016024021) approved the study. The Good Clinical Practice Unit at Aalborg and Aarhus University Hospitals monitored the study. All participants gave written informed consent.

2.2. Study design

We performed an open-label parallel group intervention study. All participants were treated with once-daily 75 mg aspirin (Hjertemagnyl®, Takeda Pharma A/S, Taastrup, Denmark) during a six-day study period. Platelet aggregation was assessed before and 1 h after ingestion of 75 mg aspirin at two separate visits: at the first day of treatment and after six days of treatment (Fig. 1).

At visit 1, blood samples were collected prior to aspirin treatment (baseline) and again 1 h after a single dose of 75 mg aspirin (acute effect). All participants then received once-daily aspirin 75 mg for six days and were instructed to ingest the last aspirin tablet 24 h before blood sampling on visit 2. After blood sampling on visit 2 (24 h after intake), participants ingested another 75 mg aspirin and the last blood sample was drawn exactly 1 h later. In order to avoid diurnal variation all examinations were performed between 8.00 am and 12.00 am, and participants were fasting from the night before blood sampling.

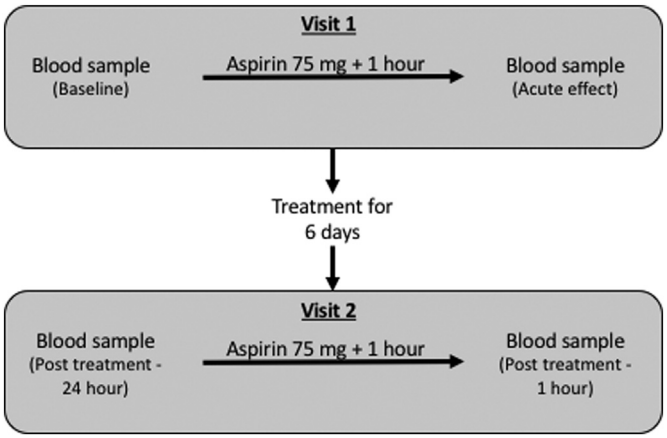

Fig. 1. Study design.

To optimize compliance, all participants received a package containing seven tablets, and the number of tablets remaining in the container was controlled at the end of the study. Compliance was further optimized by face-to-face interviews and confirmed by measurements of serum thromboxane B_2. On each visit, the investigator witnessed the ingestion of aspirin.

2.3. Laboratory investigations

2.3.1. Blood sampling

Blood samples were drawn after 30 min of rest from an antecubital vein using vacuum tubes and a 21-gauge syringe with a minimum of stasis and with patients in seated position. The first tubes were used for standard biochemical measurements.

2.3.2. Standard biochemical parameters

Haemoglobin and white blood cell count were determined by an automated haematology analyzer (Sysmex® XE-5000, Kobe, Japan). C-reactive protein and creatinine were measured in lithium-heparin plasma by the Cobas C501 Analyzer (Roche, Mannheim, Germany). HbA_{1c} was assessed by automated high performance liquid chromatography (Tosoh, Tokyo, Japan).

2.3.3. Platelet aggregation

Blood for platelet aggregation analysis was collected in 3.0 mL tubes containing hirudin and stored at room temperature for at least 30 min but no longer than 120 min before analysis. Platelet aggregation analysis was performed by multiple electrode aggregometry using the Multiplate® Analyzer (Roche, Mannheim, Germany), which is based on impedance aggregometry. Arachidonic acid 0.5 mM (AA) and thrombin-receptor-activating-peptide 32 μM/L (TRAP) were used as agonists (ASPItest and TRAPtest, Roche, Mannheim, Germany). Platelet aggregation levels are expressed as area under the curve (AUC) (aggregation units (AU) · min).

2.3.4. Platelet count and turnover

Platelet parameters were measured in the first blood sample at each visit and determined by an automated haematology analyzer (Sysmex® XE-5000, Kobe, Japan). Parameters included platelet count, immature platelet count (IPC), immature platelet fraction (IPF), mean platelet volume (MPV), platelet distribution width (PDW), platelet large-cell-ratio (P-LCR), and the highly fluorescent immature platelet fraction (H-

IPF).

2.3.5. Serum thromboxane B_2

Non-anticoagulated blood was stored for 60 min at 37 °C. Serum was then separated by centrifugation at 2600*g* for 10 min and stored at − 80 °C until analysis. Serum thromboxane B_2 was measured by ELISA (Cayman Chemical, MI, USA) according to the manufacturer's instructions. All measurements were performed in duplicates and a coefficient of variation < 20% was accepted.

2.4. Statistical analysis

All data were tested for normality using qq-plots. Normally distributed continuous variables are presented as mean ± standard deviation (SD). Categorical variables are presented as frequencies and percentages. In accordance with our matched design, paired *t*-test and Chi-square test were used to test for differences in baseline characteristics.

Platelet aggregation induced by AA and TRAP was evaluated using a mixed model analysis. This model was used to test differences in platelet aggregation within groups and between groups.

Paired *t*-tests were used in analysis of secondary endpoints (platelet turnover and serum thromboxane B_2). Pearson's correlation was used to test for correlation. The association between platelet parameters and platelet aggregation was tested for possible interaction with diabetes.

2.5. Sample size calculation

Our primary endpoint was the difference in platelet aggregation between 1 and 24 h after ingestion of aspirin. The mean change in platelet aggregation using AA in patients with T2DM and coronary artery disease has previously been reported to be 70 AU·min with a standard deviation (SD) of 97 AU·min [15]. With a minimal relevant difference of 70 AU·min, a level of significance of 5% (alpha) and a power of 90%, (1-beta) we had to include 21 patients with T2DM. Furthermore, we included 21 controls without diabetes.

Statistical analyses were performed using GraphPad Prism® version 7.0 (GraphPad Software, CA, USA) and Stata software version 13 (StataCorp, College Station, TX, USA).

3. Results

Clinical and biochemical baseline characteristics are summarized in Table 1. The matching was complete with regard to sex, whereas patients with T2DM were on average 1.4 ± 2.9 years younger than their matched control. Patients with T2DM had a mean HbA_{1c} of 53 ± 12 mM, and the use of antihypertensive and lipid-lowering drugs were more frequent in patients with T2DM than in controls.

All participants completed the study, however one blood sample from a control person could not be obtained on visit 1 due to technical problems. Pill counting and interviews did not reveal any non-compliant participants, and excellent compliance was confirmed by serum thromboxane B_2 ranging from 0.03–6.70 ng/mL corresponding to ≥ 95% inhibition of platelet COX-1 activity [17].

3.1. Platelet aggregation

Table 2 shows platelet aggregation results. At visit 2, the mean aggregation level 24 h after aspirin ingestion was significantly higher than the mean aggregation level 1 h after ingestion in both groups when using AA as agonist (Fig. 2). Patients with T2DM had an increase of 85 ± 101 AU·min ($p < 0.001$) and controls 80 ± 105 AU·min ($p < 0.001$) during the dosing interval. At baseline, AA-induced platelet aggregation was significantly higher in patients with T2DM compared to controls (Table 2 and Fig. 3).

At visit 1, aspirin acutely reduced AA-induced platelet aggregation in both patients and controls, and a further significant reduction in platelet aggregation was observed after six days of aspirin treatment (Fig. 4).

Neither the acute nor the chronic antiplatelet effect of aspirin was different between the two groups, and there was no difference in aggregation levels between patients and controls at visit 2 ($p > 0.05$).

TRAP-induced platelet aggregation was measured to evaluate platelet aggregation independently of aspirin. Accordingly, TRAP-induced aggregation levels were not affected by aspirin treatment and were similar in all blood samples within groups. Mean aggregation levels were numerically higher in patients with T2DM than controls, although statistical significance was not reached for all comparisons ($p = 0.01$ to 0.08).

Table 1
Clinical and biochemical characteristics of the study population.

Characteristics	T2DM (n = 21)	Controls (n = 21)	p value
Age (years)	61 ± 9	62 ± 9	
Male gender n (%)	14 (67)	14 (67)	
Duration of diabetes (years)	9 ± 8	–	N/A
Body mass index (kg/m^2)	30 ± 4	27 ± 5	0.10
Current smokers n (%)	3 (14)	3 (14)	1.00
Systolic blood pressure (mm Hg)	140 ± 20	132 ± 15	0.19
Diastolic blood pressure (mm Hg)	81 ± 8	81 ± 8	0.98
Heart rate (beats/min)	68 ± 11	59 ± 7	0.01
White blood cell count (10^9/L)	6.5 ± 1.3	5.8 ± 1.8	0.16
B-Haemoglobin (mM)	8.8 ± 0.8	8.8 ± 0.7	0.92
B-Haemoglobin A_{1c} (mmol/mol)	53 ± 12	39 ± 3	< 0.001
P-Creatinine (μM)	78 ± 17	80 ± 16	0.72
P-C-reactive protein (μM)	3.6 ± 4.0	2.4 ± 2.5	0.25
Medication			
Oral antidiabetics n (%)	16 (76)	–	N/A
Insulin n (%)	7 (33)	–	N/A
GLP-1 analogues n (%)	7 (33)	–	N/A
Antihypertensive drugs n (%)	16 (76)	7 (33)	0.01
Lipid-lowering drugs n (%)	11 (52)	1 (5)	< 0.001

Data are expressed as mean ± SD or number of patients (%).
T2DM, type 2 diabetes mellitus; N/A, not applicable.

Table 2
Aggregation levels in patients with type 2 diabetes and controls.

	T2DM (n = 21)	Controls (n = 21)	p value
Aggregation levels, AA 0.5 mM			
Baseline (AUC)	949 ± 159	835 ± 194	0.03
Acute effect[a] (AUC)	731 ± 256	634 ± 218	0.16
Post treatment, 1 h (AUC)	172 ± 101	215 ± 86	0.16
Post treatment, 24 h (AUC)	257 ± 108	295 ± 135	0.29
Mean difference between 1 h and 24 h (AUC)	85 ± 101 (p < 0.001)	80 ± 105 (p < 0.001)	0.88
Aggregation levels, TRAP 32 μM/L			
Baseline (AUC)	1104 ± 160	1005 ± 193	0.06
Acute effect[a] (AUC)	1110 ± 213	1000 ± 205	0.08
Post treatment, 1 h (AUC)	1141 ± 153	990 ± 177	0.01
Post treatment, 24 h (AUC)	1127 ± 163	1010 ± 197	0.03

Data are expressed as mean ± SD. All differences are evaluated using a mixed model analysis.
T2DM, type 2 diabetes mellitus; AA, arachidonic acid; TRAP, thrombin-receptor-activating-peptide; AUC, area under the curve.
[a] Controls n = 20

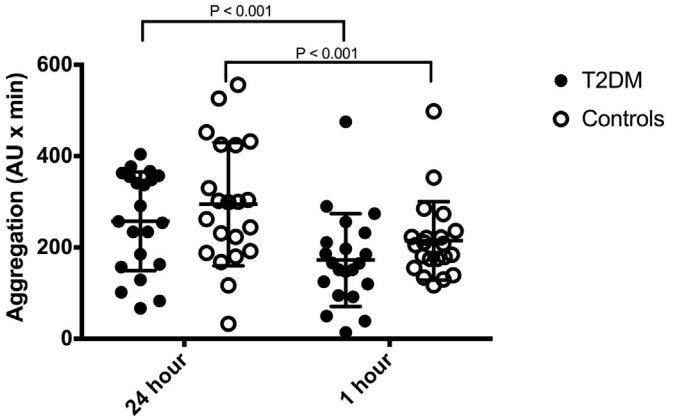

Fig. 2. Platelet aggregation after 6 days of treatment.
Platelet aggregation 1 and 24 h after intake of aspirin 75 mg using arachidonic acid as agonist in patients with type 2 diabetes and controls after 6 days of aspirin treatment.
T2DM, type 2 diabetes mellitus.

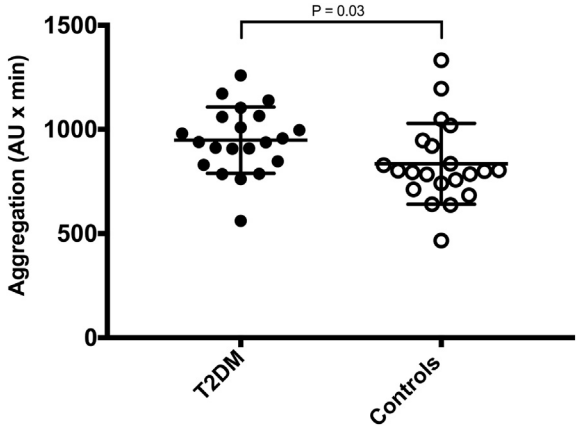

Fig. 3. Baseline platelet aggregation.
Baseline platelet aggregation levels in patients with type 2 diabetes and controls using arachidonic acid 0.5 mM as agonist.
T2DM, type 2 diabetes mellitus.

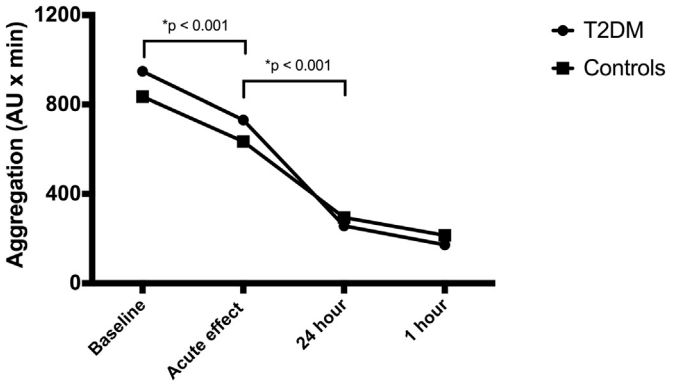

Fig. 4. Aggregation levels in patients with type 2 diabetes and controls.
Aggregation levels at visit 1 (baseline and acute effect) and visit 2 (1 h and 24 h) in patients with type 2 diabetes and controls using arachidonic acid 0.5 mM as agonist.
*The shown p-values accounts for both patients and controls.
T2DM, type 2 diabetes mellitus.

3.2. Platelet turnover

As shown in Table 3, most platelet parameters (IPF, PDW, MPV, P-LCR, H-IPF and platelet count) were similar in the two groups at baseline. However, patients with T2DM had numerically higher IPC than controls (p = 0.09), though non-significant.

Table 3
Baseline platelet parameters.

	T2DM (n = 21)	Controls (n = 21)	p value
Platelet count (10^9/L)	259 ± 61	237 ± 58	0.19
IPC (10^9/L)	8.0 ± 4.8	5.9 ± 2.3	0.09
IPF (%)	3.0 ± 1.3	2.6 ± 1.2	0.34
PDW (fL)	12.2 ± 1.7	11.7 ± 1.7	0.45
MPV (fL)	10.4 ± 0.8	10.2 ± 0.9	0.54
P-LCR (ratio)	0.282 ± 0.067	0.265 ± 0.074	0.49
H-IPF (%)	1.0 ± 0.4	0.9 ± 0.3	0.22

Data are expressed as mean ± SD.
T2DM, type 2 diabetes mellitus; IPC, immature platelet count; IPF, immature platelet fraction; PDW, platelet distribution width; MPV, mean platelet volume; P-LCR, platelet large-cell-ratio; H-IPF, highly fluorescent immature platelet fraction.

In both patients and controls baseline AA-induced platelet aggregation correlated positively with IPC (r = 0.50, p = 0.02, and r = 0.45, p = 0.04 respectively) as well as platelet count (r = 0.54, p = 0.01, r = 0.62, p < 0.01), with no differences between patients and controls (test for interaction p = 0.23 and p = 0.41 respectively).

In blood samples obtained 24 h after aspirin ingestion, a positive correlation was observed between IPC and platelet aggregation levels in the controls, but not in patients with T2DM (Controls; r = 0.45, p = 0.04, Patients; r = −0.04, p = 0.86, test for interaction p = 0.03).

No platelet parameters correlated with the changes in platelet aggregation levels between 1 and 24 h.

In both groups, platelet parameters remained stable from visit 1 to visit 2 and we did not find any statistically significant changes in any platelet parameters after aspirin treatment when compared with baseline (data not shown).

3.3. Serum thromboxane B_2

Four samples were excluded due to high coefficients of variation, one sample from a control and three samples from patients.

At baseline, serum thromboxane B_2 level was similar in the two groups (p = 0.63). The first dose of aspirin reduced the serum thromboxane B_2 level, and it was further reduced after six days of treatment.

At visit 2, the mean serum thromboxane B2 level 24 h after aspirin ingestion was significantly higher than the mean level 1 h after ingestion in both patients (4.27 ± 3.03 vs. 1.87 ± 1.74 ng/mL, p < 0.001) and controls (4.05 ± 2.87 vs. 1.63 ± 1.22 ng/mL, p < 0.001).

4. Discussion

We observed increased platelet aggregation levels at the end of the 24-hour dosing interval of aspirin in patients with T2DM without a history of CVD and age- and sex-matched controls. Furthermore, the study confirmed that aspirin naïve patients with T2DM have increased platelet aggregation compared to non-diabetic matched controls. Platelet aggregation was acutely reduced after the first dose of aspirin, and after six days of treatment, aggregation levels were substantially further reduced. Finally, we found similar platelet parameters in the two groups, although a trend toward an increased platelet turnover was found in patients with T2DM compared to controls.

Previous studies have reported a reduced antiplatelet effect of aspirin in patients with T2DM compared to non-diabetic controls [15,18–24]. However, most studies included patients with a history of CVD [15,19–23]. Only one study has investigated platelet aggregation levels in patients with T2DM without a history of CVD [24]. However, this study compared T2DM patients without a history of CVD with non-diabetic survivors of myocardial infarction. Both groups received

aspirin monotherapy, and T2DM patients had higher on-treatment aggregation than patients without diabetes [24].

Studies in patients with diabetes and a history of CVD have also shown higher on-treatment platelet aggregation than patients without diabetes. This has been reported in patients receiving aspirin monotherapy [19–21] or dual antiplatelet therapy with aspirin and clopidogrel [20].

Our study differs from these studies by having a homogeneous population of T2DM without CVD and a control group consisting of sex- and age-matched volunteers without diabetes.

In accordance with previous studies [15,25–28], our study showed that patients treated with low-dose aspirin have a time-dependent increase in platelet aggregation through the dosing interval. Our study extends these findings to a well-controlled (HbA_{1c} = 53 ± 12 mmol/mol) population of patients with T2DM and no previous history of CVD. In our study, an increase in aggregation was also seen in the control group during the 24-hour dosing interval, and the increase was similar in T2DM patients and controls. The observed increase in aggregation could be more clinically relevant in patients with diabetes, since platelets are activated through many different pathways, and aspirin only inhibits the thromboxane A_2 pathway. In a previous study it was shown, that a high level of platelet aggregation despite antiplatelet treatment was associated with increased risk of long-term adverse cardiovascular events in patients with T2DM and coronary artery disease [29]. Thus, perhaps the platelet dysfunction observed in patients with T2DM makes these patients more vulnerable to the reduced platelet inhibition at the end of the 24-hour dosing interval.

Different approaches have been suggested to improve the effect of aspirin, including a twice-daily dosing [23,25,30–32] or an increased dose [23,30,32]. Capodanno et al. showed that low-dose aspirin given twice daily reduced platelet aggregation more than low-dose aspirin given once daily in patients with T2DM and CVD [23]. Additionally, the authors did not observe any dose effect on platelet aggregation when doubling the dose from 81 mg to 162 mg once daily. This may indicate that increasing the frequency of aspirin administration has greater antiplatelet effect compared to increasing the dose. Only one of the studies was conducted in patients with T2DM without CVD [32]. This study similarly reported that 100 mg aspirin given twice daily reduced platelet aggregation more effectively than 100 mg once daily assessed by both the VerifyNow™ Aspirin and whole blood aggregometry using the Multiplate® Analyzer [32]. So far, the studies investigating a twice-daily dosing regimen have had small sample sizes and only evaluated laboratory-assessed outcomes. Whether a twice-daily dosing regimen has any clinical benefits in CVD prevention remains to be elucidated. The safety profile of this dosing strategy also needs evaluation in large randomized controlled trials.

To our knowledge, this is the first study investigating platelet response to aspirin in aspirin-naïve T2DM subjects without a history of CVD. This has further allowed us to assess baseline platelet aggregation. We observed a higher platelet aggregation when using AA as agonist in patients with T2DM compared to controls, confirming alterations in platelet function in this patient group. A previous study found no differences between T2DM patients and non-diabetic controls when investigating markers of platelet activation [33], however platelet aggregation was not assessed. Platelets in T2DM patients are often characterized as hyperreactive, but this term is poorly defined and often refers to high on-treatment platelet reactivity [12]. Our study demonstrates that the increased platelet aggregation was present before treatment with antiplatelet drugs and to our knowledge this has not been shown before.

Patients presenting with acute ischaemic events are recommended a high loading dose of aspirin typically ranging from 150 to 300 mg in order to inhibit platelet aggregation sufficiently [34]. After the first dose of low-dose aspirin, we observed a reduction in platelet aggregation, however after six days of treatment, a further substantial reduction was observed. This showed that the first dose of low-dose aspirin was not sufficient to provide complete platelet inhibition. Our results are in line with a previous study [35] in which 81 mg of aspirin did not suppress thromboxane B_2 synthesis sufficiently on the first day of administration and a dose of at least 162 mg was needed to exert an immediate antiplatelet effect. We did not test if a higher loading dose of aspirin at the first administration was able to inhibit platelet aggregation more than the acute effect that we observed after the first administration. Our results, however, demonstrate the importance of a longer treatment period before evaluation of platelet aggregation when using low-dose aspirin. It seems that the platelet inhibitory effect of aspirin increased progressively from the first administration to the 6th day. A treatment period longer than 6 days might have suppressed platelet aggregation levels even further.

Different theories have been proposed to explain platelet dysfunction in diabetic patients, including hyperglycaemia, insulin resistance and higher platelet turnover [12]. In the present study, baseline platelet aggregation correlated positively with IPC, a measure often used as a surrogate marker of platelet turnover. T2DM patients had a trend toward higher IPC than controls, though non-significant. This may partially explain the higher aggregation levels in patients with T2DM, since newly formed platelets are considered to be more sensitive to activation and aggregation compared to mature platelets [12]. Increased platelet turnover has previously been associated with reduced antiplatelet effect of aspirin [9,10]. However, in our study, none of the investigated platelet parameters correlated to the difference in platelet aggregation 1 and 24 h after ingestion of aspirin.

Serum thromboxane B_2 also increased during the dosing interval, which corresponds to the findings of increased platelet aggregation at the end of the 24-hour dosing interval. Rocca et al. [36] also demonstrated an increase in serum thromboxane B_2 in both patients with and without diabetes treated with low-dose aspirin. In our study, it should be noted that the level of serum thromboxane B_2 was low after 24 h, however the increase in thromboxane B_2 during the dosing interval still indicated recovery of platelet COX-1 activity.

The present findings are of considerable clinical interest in the light of the on-going discussion of aspirin's role in primary prevention in patients with T2DM. Currently, no studies have provided convincing evidence to support the routine use of aspirin for primary prevention of CVD in patients with T2DM [4]. Results of the on-going studies ACCEPT-D (Aspirin and Simvastatin Combination for Cardiovascular Events Prevention Trial in Diabetes [37]) and ASCEND (A Study of Cardiovascular Events in Diabetes, NCT00135226) will hopefully establish whether patients with diabetes benefits from once daily dosing of aspirin. If these studies show a net benefit from aspirin treatment in the primary prevention of CVD in patients with T2DM, our study, however, indicates that this patient group may achieve additional benefit from an alternate dosing regimen. Different treatment options may be twice daily dosing of low-dose aspirin or perhaps once daily with intake at bedtime instead of in the morning [38,39].

Strengths of this study include the well-characterized study population, the thorough validation of compliance and the standardized study design with specific timing of blood sampling in relation to aspirin ingestion. Furthermore, we included an age- and sex matched control group. The use of aspirin naïve subjects allowed us to obtain baseline platelet aggregation level and to investigate the acute effects of aspirin. Further, our group of T2DM patients are representative of patients with diabetes without CVD when it comes to age, medical treatment and duration of diabetes. The study is limited by a relatively small number of patients included and the results should be confirmed in a larger patient sample. A further limitation is that the study was too small to thoroughly address correlations between platelet aggregation and parameters of platelet turnover. We cannot exclude that differences in concomitant medications (e.g. statins, therapy for diabetes) could have affected platelet reactivity. However, the medication of the patients with diabetes reflects “standard of care treatment” and, thus, the everyday clinical situation.

In conclusion, aspirin-treated patients with T2DM without a history of CVD had increased platelet aggregation at the end of the standard 24-hour dosing interval of aspirin, but the increase was not different from the increase observed in matched controls. Further, aspirin-naïve T2DM patients had increased platelet aggregation compared to non-diabetic matched controls, confirming alterations in platelet function in patients with T2DM.

Conflict of interest statement

We confirm that there are no financial or other conflicts of interest for any of the authors.

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