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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.

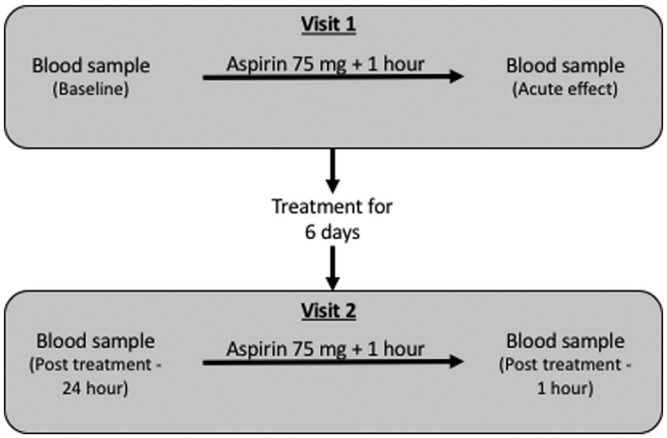

Fig. 1. Study design.

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-