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24-hour antiplatelet effect of aspirin in patients with previous definite stent thrombosis

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ABSTRACT

Objective: Once-daily aspirin is standard treatment, but recent studies point towards increased platelet function at the end of the dosing interval. Stent thrombosis (ST) has been linked with reduced antiplatelet effect of aspirin, so we investigated if platelet inhibition by aspirin declines through 24 h in patients with previous definite ST. Furthermore, we explored whether increased levels of immature platelets and thrombopoietin are associated with a particularly rapid recovery of platelet function.

Methods: This case–control study included 50 patients with previous definite ST matched with 100 patients with stable coronary artery disease and 50 healthy volunteers. All participants were on aspirin 75 mg/day mono antiplatelet therapy. Platelet aggregation was measured 1 and 24 h after aspirin intake using platelet aggregometry (Multiplate® Analyzer). Cyclooxygenase-1 activity, platelet activation, immature platelets, and thrombopoietin were measured.

Results: Platelet aggregation increased by 109 ± 150 (arachidonic acid) and 47 ± 155 (collagen) aggregation units per minute from 1 to 24 h after aspirin intake (p-values <0.0001) with corresponding increases in thromboxane B_2 (5.6 ± 5.1 ng/ml, $p < 0.0001$) and soluble P-selectin (6.2 ± 15.5 ng/ml, $p < 0.0001$). Platelet aggregation increased equally in all groups, but patients with previous ST displayed the highest levels of platelet aggregation at 24 h (p-values ≤ 0.05) and the highest levels of immature platelets ($p < 0.01$) and thrombopoietin ($p < 0.0001$).

Conclusions: Platelet inhibition declined significantly during the 24-hour dosing interval in aspirin-treated patients with previous definite ST or stable coronary artery disease and in healthy individuals. Increased levels of immature platelets and thrombopoietin were observed in patients with previous definite ST.

1. Introduction

Low-dose aspirin reduces by one-fifth the risk of recurrent arterial thrombosis in patients with coronary artery disease (CAD), and international clinical guidelines recommend that low-dose aspirin is administered once daily in these patients [1]. This treatment strategy reflects that aspirin is considered to sustain adequate platelet inhibition through the 24-hour dosing interval. However, emerging evidence questions this assumption. Recent studies point towards a gradual attenuation of aspirin's antiplatelet effect through 24 h causing increased residual platelet aggregation [2–5] and cyclooxygenase (COX) activity [4–6]. Furthermore, pharmacodynamic studies suggest that a twice-daily dosing regimen provides more consistent platelet inhibition through 24 h than does the recommended once-daily regimen [7–9].

Stent thrombosis (ST) is a devastating complication of percutaneous coronary intervention [10], and the complex pathophysiology of in-stent thrombus formation includes procedural and pharmacological factors [11]. Antiplatelet drugs are essential in maintaining stent patency and preventing stent occlusion [11]; however, in patients with a history of definite ST residual platelet aggregation during aspirin treatment is substantial [12,13]. Patients with previous definite ST also seem to have an accelerated platelet turnover as indicated by higher levels of immature platelets [12]. Immature platelets are large, reticulated and highly reactive, which renders them prone to thrombus formation. Moreover, given aspirin's plasma half-life of only 20 min, newly formed platelets may produce thromboxane due to uninhibited COX-1 activity.

Abbreviations: CAD, coronary artery disease; COX, cyclooxygenase; ST, stent thrombosis; TXB_2, thromboxane B_2.

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Recent studies have associated an accelerated platelet turnover with increased residual platelet aggregation in different cardiovascular settings [12,14–17]. Furthermore, differences in platelet production may contribute to variability in residual platelet aggregation levels because thrombopoietin, the primary hormonal regulator of platelet production, directly affects platelet aggregability [18].

We hypothesized that platelet inhibition by low-dose aspirin is reduced during the standard 24-hour dosing interval. Based on previous studies, this may particularly be the case in patients with previous definite ST. Moreover, we explored whether the recovery of platelet aggregation is particularly pronounced in patients with an accelerated platelet turnover.

2. Methods

The study was conducted in accordance with the Helsinki II Declaration, and the study protocol was approved by the Central Denmark Region Committees on Biomedical Research Ethics (#2011-0231). Written informed consent was obtained from all participants, of which only healthy volunteers were remunerated for their participation.

2.1. Design and study population

We performed a case–control study comprising 200 participants included from March to December 2012; 50 patients with previous definite ST, 100 patients with stable CAD and 50 healthy individuals. All participants were on mono antiplatelet therapy with aspirin 75 mg once daily during study participation, *i.e.* no other antithrombotic drugs (including ADP receptor antagonists) were allowed.

Patients with previous definite ST were identified according to the Academic Research Consortium criteria [19] and underwent index percutaneous coronary intervention on three interventional centres in Denmark between April 2001 and June 2009. Subsequent diagnoses of definite ST were adjudicated by an independent specialist committee on the basis of coronary angiographies as previously described [20]. The recruitment of patients with previous definite ST is outlined in Fig. 1. After including the ST patients, we matched these 1:2:1 at an individual level with stable CAD patients and healthy individuals. All three groups were matched on age (±one year) and sex, and ST patients and CAD patients were further matched on diabetic status.

Stable CAD patients were identified in the Western Denmark Heart Registry. This registry collects patient and procedure data from all interventional centres in the western part of Denmark. Stable CAD patients were defined as patients previously undergoing percutaneous coronary intervention with stenting, but with no history of myocardial infarction. Healthy individuals were recruited by advertising in the local media.

Exclusion criteria for ST patients were aspirin intolerance, acute disease, use of anticoagulants or any drugs (except aspirin) affecting platelet function (including ADP receptor antagonists and non-steroidal anti-inflammatory drugs), alcohol intake the day before blood sampling, platelet count $<120 \times 10^9$/l and any ischemic event or revascularization procedure (percutaneous coronary intervention or coronary artery bypass grafting) within the last six months. Exclusion criteria for stable CAD patients were the same, except that no history of myocardial infarction was accepted. Healthy individuals were defined as drug-abstinent individuals with no previous or ongoing systemic disease.

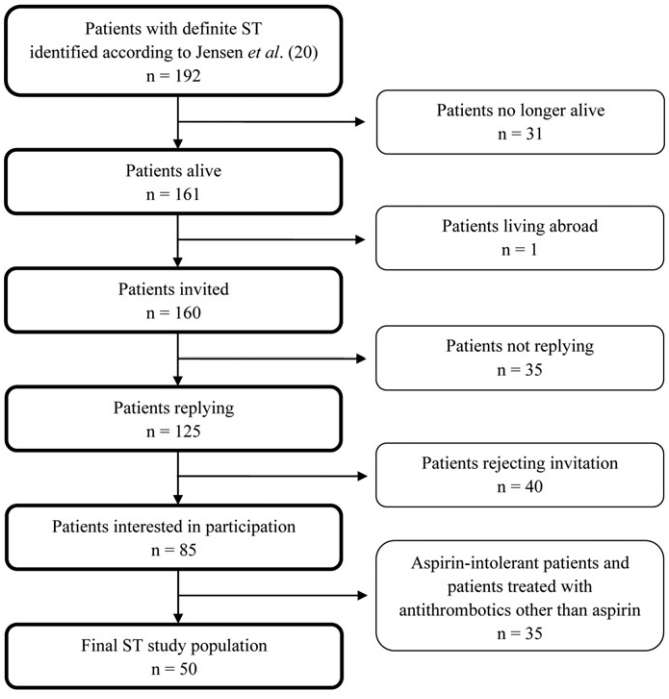

Fig. 1. Flow diagram showing the inclusion of patients with previous definite ST. ST = stent thrombosis.

Standardized blood sampling was performed twice in every patient; 1 h and 24 h after aspirin intake. Samples were drawn from an antecubital vein into evacuated tubes through a 19-gauge butterfly needle.

2.2. Platelet aggregometry

Multiple electrode aggregometry was performed using the Multiplate® Analyzer (Roche Diagnostics International LDT, Rotkreuz, Switzerland). Multiplate® Analyzer is an impedance aggregometer detecting differences in electrical resistance between two electrodes in whole blood anticoagulated with hirudin [21]. Arachidonic acid and collagen were used as agonists at final concentrations of 1.0 mM and 3.2 μg/ml, respectively. Agonists were stored in temperature monitored refrigeration units and allowed to reach room temperature prior to reconstitution. Analyses were performed between 30 and 120 min after blood sampling, and aggregation was reported as area under the curve (aggregation units × minute).

2.3. Study medication and compliance

ST patients and stable CAD patients were on permanent aspirin mono antiplatelet therapy upon study enrollment. In order to optimize compliance and avoid pharmacokinetic heterogeneity, all participants (including healthy volunteers) received a dosage box containing six tablets of 75 mg non-enteric coated aspirin (Hjerdyl®; Sandoz, Copenhagen, Denmark), *i.e.* one tablet for each of the last six days prior to blood sampling. A laboratory technician witnessed the intake of a seventh aspirin tablet on the day of blood sampling. Compliance was confirmed by serum thromboxane B_2 (TXB_2) measurements. Co-medication was ascertained on the day of blood sampling and confirmed by reviewing hospital records.

2.4. Serum thromboxane B_2, soluble P-selectin and thrombopoietin

Serum TXB_2 was used as a measure of COX-1 activity and determined using an enzyme-linked immunosorbent assay (Cayman Chemical, Ann Arbor, MI, USA) as previously described [22]. Soluble serum P-selectin, a marker of platelet activation, and thrombopoietin were determined using enzyme-linked immunosorbent assays (R&D Systems Europe, Abingdon, UK) according to the manufacturer's instructions.

2.5. Platelet turnover

The absolute and relative numbers of immature platelets (immature platelet count and immature platelet fraction) as well as the mean platelet volume were used as markers of platelet turnover. Complete blood counts were assessed using automated flow cytometry (Sysmex XE-5000; Sysmex Kobe, Japan) as previously described [23].

2.6. Statistical analysis and sample size

Summary statistics and frequencies were generated using the Stata 12.1 software package (Stata Corp LP, TX, USA), and graphics were performed in GraphPad Prism version 6.03 (GraphPad Software, La Jolla, CA, USA). Continuous variables are presented as mean ± standard deviation or median (interquartile range) and dichotomous variables are presented as numbers and percentages. Distributions of discrete variables were compared with the χ^2 test. Comparisons of continuous variables between two groups were performed with an unpaired *t* test or the two-sample Wilcoxon rank-sum test as appropriate. Comparisons of continuous variables measured 1 versus 24 h after aspirin intake were performed with a paired *t* test. Comparisons of continuous variables across three groups were performed with one-way ANOVA followed by Tukey's *post-hoc* correction for pairwise comparisons, except that the Kruskal–Wallis test was used if the assumption of equal variance was not met. Where appropriate, calculations were made using log-transformed data. Spearman's rank correlation coefficient was calculated for bivariate analyses. Multiple linear regression analyses (including age, sex, body mass index, and smoking) were used to identify independent determinants of platelet aggregation. Platelet aggregation was analyzed retaining the original scaling of continuous variables to preserve statistical power and to minimize the risk of type II errors. Two-sided *p*-values below 0.05 were considered significant.

We conducted a pilot study including 29 aspirin-treated patients with stable CAD to estimate the effect size. The mean difference in platelet aggregation from 1 to 24 h after aspirin intake was 82 ± 170 aggregation units × minute when using arachidonic acid as agonist. With 190 participants recruited and a two-sided alpha level of 5%, the study had 90% statistical power to detect a 40 aggregation units × minute mean difference in platelet aggregation between samples taken 1 versus 24 h after aspirin intake. Assuming a dropout rate of 5%, we enrolled a total of 200 participants.

A *post hoc* dropout analysis was performed to evaluate potential selection bias introduced during the inclusion of ST patients. We compared age and sex of invited ST patients not included in the study with those actually included (Fig. 1). We found no differences indicating that results were not biased by a skewed inclusion of ST patients.