Thrombosis Research 143 (2016) 58–62

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Fibrin clot formation and fibrinolysis in patients with a history of coronary stent thrombosis

Thea C. Godschalk [a,*,1], Joke Konings [b,1,2], José W. Govers-Riemslag [b], Jurriën M. ten Berg [a], Christian M. Hackeng [c], Hugo ten Cate [b]

[a] St Antonius Center for Platelet Function Research, Department of Cardiology, St. Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands
[b] Laboratory for Clinical Thrombosis and Haemostasis, Departments of Internal Medicine and Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands
[c] St Antonius Center for Platelet Function Research, Department of Clinical Chemistry, St. Antonius Hospital, Koekoekslaan 1, 3435 CM Nieuwegein, The Netherlands

ARTICLE INFO

Article history:
Received 8 March 2016
Received in revised form 29 April 2016
Accepted 30 April 2016
Available online 3 May 2016

Keywords:
Coronary thrombosis
Fibrin
Fibrinolysis
Percutaneous coronary intervention
Stent

ABSTRACT

Introduction: Coronary stent thrombosis is a devastating complication of percutaneous coronary intervention (PCI). Multiple factors underlie the pathophysiological mechanisms of stent thrombosis. Previous studies demonstrated that patients with stent thrombosis, compared to control PCI patients, formed denser fibrin clots in vitro which were more resistant to fibrinolysis, suggesting that altered fibrin clot properties may contribute to the pathophysiology of stent thrombosis. We assessed the plasma fibrin clot formation and fibrinolysis of patients with and without stent thrombosis.
Methods: Cases (patients with stent thrombosis) and matched controls (patients without stent thrombosis) were included for a matched case-control study. Matching was performed on indication and time of the index PCI (initial stent implantation) from the cases. Fibrin clot formation and fibrinolysis were assessed in vitro by turbidimetric assays, with human thrombin to initiate fibrin polymerization and tissue type plasminogen activator to initiate fibrinolysis. Lag time, maximal absorbance and clot lysis time were determined by these assays.
Results: In total, 27 cases and 27 controls were included. No significant differences were observed between cases and controls in lag time (173 vs. 162 s, $p = 0.18$), maximal absorbance (0.78 vs. 0.83, $p = 0.36$), and clot lysis time (69 vs. 71 min, $p = 0.78$). Fibrin clot formation and fibrinolysis were not associated with stent thrombosis.
Conclusions: Plasma fibrin clot formation and fibrinolysis were not significantly different between patients with stent thrombosis and matched control patients, suggesting that fibrin clot formation and fibrinolysis play no significant role in the pathophysiology of stent thrombosis.

1. Introduction

Coronary stent thrombosis is a feared complication of percutaneous coronary intervention (PCI). Around 80% of the patients with stent thrombosis present with a myocardial infarction [1,2], and stent thrombosis is associated with mortality rates between 10 and 40% [3–5]. The incidence of stent thrombosis is approximately 1–4%, despite dual antiplatelet therapy with aspirin and clopidogrel [3,6]. Multiple factors underlie the pathophysiological mechanisms of stent thrombosis, such as stent underexpansion and high residual platelet reactivity [7,8].

Thrombus formation includes the interplay of platelet activation and aggregation with fibrin clot formation. Clot stability is largely determined by mechanical strength and sensitivity to fibrinolysis. Previous studies by Undas and co-workers [9,10] demonstrated that patients with stent thrombosis, compared to control PCI patients, formed denser fibrin clots which were more resistant to fibrinolysis. In these studies, 90% of the patients received a bare metal stent (BMS). However, the use of drug eluting stents (DES) compared to BMS has increased in recent years, which might have changed the risk factors associated with stent thrombosis.

Therefore, we assessed the potential of plasma fibrin clot formation (lag time, maximal absorbance) and fibrinolysis (clot lysis time) from patients with a history of stent thrombosis compared to matched control patients in a currently representative cohort.

Abbreviations: BMS, bare metal stent; CI, confidence interval; DES, drug eluting stent; OR, odds ratio; PCI, percutaneous coronary intervention.

* Corresponding author at: St. Antonius Hospital, P.O. Box 2500, 3430 EM Nieuwegein, The Netherlands.
E-mail addresses: t.godschalk@antoniusziekenhuis.nl (T.C. Godschalk), j.konings@thrombin.com (J. Konings), j.govers@maastrichtuniversity.nl (J.W. Govers-Riemslag), jurtenberg@gmail.com (J.M. ten Berg), c.hackeng@antoniusziekenhuis.nl (C.M. Hackeng), h.tencate@maastrichtuniversity.nl (H. ten Cate).
[1] These authors contributed equally to this project.
[2] Synapse Research Institute, Cardiovascular Research Institute Maastricht, Maastricht University, Oxfordlaan 70, 6229 EV Maastricht, The Netherlands.

http://dx.doi.org/10.1016/j.thromres.2016.04.026
0049-3848/

2. Patients and methods

2.1. Study design and population

A single-center case-control study including PCI patients with stent implantation was performed. Cases underwent an index PCI (PCI of initial stent implantation) after which they suffered from a definite stent thrombosis according to the Academic Research Consortium criteria [11]. The timing of stent thrombosis was divided into cases with early (≤30 days after index PCI) and late stent thrombosis (< 30 days after index PCI). Included cases had suffered from stent thrombosis between January 2007 and September 2011. Controls underwent an index PCI without suffering from stent thrombosis between index PCI and blood sampling. Control patients were matched 1:1 based on the indication (stable angina pectoris, unstable angina pectoris/non ST-segment elevation myocardial infarction, ST-segment elevation myocardial infarction) and time (± 14 days) of the index PCI from the cases. Subjects using oral anticoagulants or heparins at the time of blood collection were excluded. We selected all patients with stent thrombosis, who were matched with a control patient, and with available blood samples from the matched stent thrombosis and control patients.

Written informed consent was provided by all participants. The study was approved by the local institutional Ethics Committee and was conducted according to the principles of the Declaration of Helsinki.

2.2. Blood collection and preparation

All subjects had visited the St Antonius Hospital for blood sampling (Nieuwegein, The Netherlands). The minimal time interval between the last performed PCI and blood sampling was one month for cases and controls. Venous blood samples were collected from the antecubital vein using 21-gauge needles and Vacuette® tubes (Greiner Bio-one, Frickenhausen, Germany) containing 3.2% (w/v) sodium citrate. To avoid haemostatic activation, the first 5 ml of free-flowing blood was discarded. Platelet poor plasma was obtained by two separate centrifugation steps. Samples were first centrifuged for 10 min at 150*g*, followed by 15 min at 2500*g*. All platelet poor plasma samples were stored at −80 °C until analysis.

2.3. Fibrin clot formation and fibrinolysis assay

To monitor fibrin clot formation, plasma samples were diluted 1.67 times with Hepes-buffer (25 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 150 mM NaCl, pH = 7.5) and 125 μl of diluted plasma was transferred into a low binding polystyrene 96-well plate (Greiner, Frickenhausen, Germany). Fibrin polymerization was started by addition of 25 μl activation mixture. The activation mixture contained human thrombin (Enzyme Research Laboratories, Swansea, UK; final concentration: 0.75 nmol/l), phospholipids which were prepared by sonication as described earlier [12] (1,2-dioleoyl-*sn*-glycero-3-phosphoserine, 1,2-dioeoyl-*sn*-glycero-3-phosphocholine, 1,2-dioleoyl-snglycero-3-phosphoethanolamine (DOPS/DOPC/DOPE, 20/60/20, mol/mol/mol), Avanti Polar lipids Inc., Alabaster, Alabama, USA; final concentration: 10 μmol/l), and $CaCl_2$ (final concentration: 16 mmol/l). Measurements were at 405 nm every 15 s for 60 min at 37 °C using an ELx808 plate reader (Biotek Instruments, Winooski, VT). The lag time, defined as the time to an increase of 0.01 optical density from baseline, together with the maximal absorbance were determined from the curves of the turbidity measurements. Turbidity measurements were performed in duplicate.

To monitor fibrinolysis, recombinant tissue plasminogen activator (Boehringer Ingelheim, Alkmaar, the Netherlands; final concentration: 50 ng/ml) was added to the activation mixture, as described as above, at the start of the assay and turbidity was recorded for 6 h. Clot lysis time was calculated as the time from 50% clot formation to 50% fibrinolysis. Lysis measurements were performed in triplicate.

The final concentrations of human thrombin and recombinant tissue plasminogen activator used in these assays were determined by turbidimetric assays with a normal plasma pool of healthy volunteers not using any medication.

2.4. Laboratory measurements

Plasma concentrations of fibrinogen were measured using a Sysmex® CA-7000 System Automated Coagulation Analyzer with reagents obtained from Siemens Healthcare Diagnostics (Marburg, Germany) according to the Claus method [13]. Platelet count was measured using a LH 750 (Beckman Coulter) and cholesterol levels were using a Cobas 6000 (Roche Diagnostics). Hypercholesterolemia was defined as an increased level of total cholesterol (> 5.0 mmol/l), triglycerides (> 1.5 mmol/l), LDL (> 2.5 mmol/l) or a decreased level of HDL (< 1.0 mmol/l).

2.5. Statistical analysis

Statistical analyses were performed with PRISM for Windows, version 5.00 (GraphPad Software, San Diego, CA, USA), and SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Continuous data are expressed as mean ± standard deviation, and categorical data are expressed as frequencies no./total no. (%).

Baseline characteristics and laboratory measurements at the time of blood sampling were analyzed using McNemar test or paired Student's *t*-test. Differences between cases and controls for fibrin clot properties were analyzed using univariate conditional logistic regression, reported as odds ratios (OR) with 95% confidence intervals (CI). Subgroup analysis was performed for cases with early vs. late stent thrombosis, and for cases with early stent thrombosis and their matched controls, as well as for cases with late stent thrombosis and their matched controls. A two-tailed p-value < 0.05 was considered as statistically significant.

3. Results

3.1. Patients

A total of 27 cases and 27 controls were included. Twelve cases had experienced an early stent thrombosis and 15 cases a late stent thrombosis. None of the matched controls experienced a stent thrombosis between index PCI and blood sampling. Baseline characteristics are summarized in Table 1. Compared to controls, significantly more cases were current smokers at time of index PCI. The rate of hypertension was numerically higher for cases than for controls however without reaching statistical significance.

The mean time-interval between stent thrombosis and blood sampling for cases was 35 ± 25 months and between index PCI and blood sampling for controls was 42 ± 26 months. More cases than controls were on dual antiplatelet therapy at the time of blood sampling (Table 2). Fibrinogen levels, cholesterol levels and platelet count were comparable between cases and matched controls.

3.2. Fibrin clot properties

The fibrin clot properties were not significantly different between cases and controls (lag time: 173 ± 47 s vs. 162 ± 28 s, p = 0.18; maximal absorbance: 0.78 ± 0.16 vs. 0.83 ± 0.21, p = 0.36; clot lysis time: 69 ± 20 min vs. 71 ± 25 min, p = 0.78) (Fig. 1). Lag time, maximal absorbance, and clot lysis time were not associated with stent thrombosis (Table 3). Subgroup analyses for cases with early vs. late stent thrombosis showed no significant differences in fibrin clot properties (Table 4). Also, analyses of cases with early