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Journal für Kardiologie - Austrian Journal of Cardiology 2004; 11 (7-8), 322-325
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Increased Platelet Activation by PTFE-Covered Coronary Stent Grafts: A Flow Cytometric Analysis in a Pulsed Floating Model of Recirculating Human Plasma

C. Beythien, M. A. Brockmann, K. Gutensohn, C. A. Nienaber

Abstract: Various designs are under investigation to resolve the problem of acute thrombosis and restenosis after stent implantation in coronary arteries. The interaction of stent surface with both platelets and the coagulation system has been shown to play a major role in this process. To investigate effects of PTFE (Teflon) coating stents of the same design with and without PTFE cover (n = 12 for each condition) were placed in an in vitro model of recirculating human platelet-rich plasma. Samples were drawn every two min until stent thrombosis to analyze platelet activation by flow cytometry using monoclonal antibodies against activation dependent epitopes CD62p (p-selectin) and CD63 (GP33) expressed as „mean channel fluorescence intensity“ (MCFI). Additionally, time until appearance of macroscopic visible platelet aggregates, and time until stent thrombosis were measured. Flow cytometric analyses revealed a maximum expression for CD62p (MCFI 37.2 ± 5.8) after 8 min, and after 10 min for CD63 (MCFI 40.0 ± 5.0) with PTFE-coated stents, meanwhile with bare stents maximum expression of both epitopes (MCFI 38.1 ± 8.4) was reached after 12 min; coated vs. bare stents p = 0.05. PTFE-coating induced platelet aggregates after 5.7 ± 1.7 min, whereas uncoated stents after 6.8 ± 2.7 min; p = 0.04. Likewise, time until stent thrombosis was shorter in systems with PTFE-covered stents compared to uncoated stents; 11.1 ± 1.4 min versus 12.6 ± 2.2 min; p = 0.035. Thus, PTFE-coating increases in vitro thrombogenicity by activating platelets and coagulation.

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Introduction
To resolve the problem of acute stent-thrombosis and restenosis after implantation in coronary arteries, different designs and coatings are currently under investigation to improve the interaction of foreign surface with platelets and the coagulation system. The Jostent® coronary stent graft (JOMED/SITOMed) with expandable polytetrafluoro-ethylene (PTFE, Teflon) graft material is a new stent design previously used in the treatment of aortocoronary vein graft lesions, and coronary aneurysms [1–4]. To investigate the PTFE covered stents and their interaction with platelets and the coagulation system, we utilized an in vitro model of recirculating human platelet-rich plasma to monitor (1) stent induced expression of activation dependent glycoproteins on platelet surface, (2) the time until the occurrence of macroscopic visual platelet aggregates, and (3) the time until stent thrombosis. The results were compared to the characteristics of the identical stainless steel (316L) stent without PTFE cover.

Methods
To quantify stent induced platelet activation the expression of glycoproteins were measured in an previously described in vitro model [5–7]. Experiments were conducted on blood from 12 obviously healthy, non-smoking male volunteers (age 29 ± 5 years) without any medication during and/or 14 days prior to blood collection. Forty ml of blood was collected via 16G needles from large antecubital veins in plastic syringes filled with sodium citrate (1:10) without the use of a tourniquet. Platelet rich plasma (PRP) was prepared by centrifugation of the whole blood at 300 g for 10 min at room temperature (20 ± 2 °C). After removal of the PRP, the remaining plasma was centrifuged at 2500 g for 10 minutes at room temperature to prepare platelet-poor plasma (PPP). Using a Coulter Counter STKR® (Beckman-Coulter, Fullerton, CA, USA) the platelet count within PRP was measured, and the PRP was diluted with PPP to a final concentration of 250 plt/nl. Commercialized PTFE covered and uncoated stainless steel stents with a length of 12 mm in expanded condition were mounted on a conventional polyethylene percutaneous transluminal balloon angioplasty catheter (Boston Scientific, Watertown, MA, USA), and implanted into silicon tubing with 12 atm for 30 s to a final diameter of 4 mm to simulate in-human situations. Then PRP was carefully filled into the tubing systems. After recalcification to physiological concentrations PRP was rotated by a roller pump (Ismatec, Zurich, Switzerland) with a flow rate of 8 ml/min., and a flow velocity of 2 cm/s. The temperature of the in vitro tubing systems was...
kept stable at 37 °C by a water bath. In three parallel silicon tubings one system was equipped with a PTFE covered stent, one with uncovered stent, and one without stent (control).

Aliquots of 100 µl PRP were removed before the circulation was started and after 2, 4, 6, 8, 10, 12, and 14 min. The samples were immediately fixed with 0.15 M phosphate-buffered saline (PBS; Gibco BRL, Eggenstein, Germany) containing glyoxal 0.2 % w/v (Merck, Darmstadt, Germany), and paraformaldehyde 0.4 % w/v (Serva, Heidelberg, Germany). Stabilization was performed by dilution 1:10 with PBS containing 0.2 % w/v Glycine (Serva, Heidelberg, Germany), labeled directly with monoclonal antibodies, and then stored at +4 °C in the dark [8]. Monoclonal antibodies CD41a, CD62p, and CD63 (Immunotech, Hamburg, Germany) were used. Flow cytometry analysis was performed within 2 hrs on a FACScan® cytometer (Becton Dickinson, Mountain View, USA). A life gate was set around CD41a positive cells; only those cells expressing this platelet specific membrane protein were included, and 20,000 events were analyzed. Results were expressed as „mean channel fluorescence intensity“ (MCFI). Antibody positive cells were defined as cells with fluorescence higher than isotype control.

In addition, time until stent thrombosis, and macroscopic visible platelet aggregates was measured.

Statistical analysis

Data comparison over the course of flow cytometric analysis were performed using the Friedman test. Statistical comparisons were performed with „Student’s t-Test“ for paired data; p values of 0.05 or less were considered to be statistically significant. All values indicated are mean ± standard error of mean.

Results

Flow cytometric analyses

After starting of circulation the expression of monoclonal antibodies increased with a maximum after 8 min for CD62p, and after 10 min for CD63 in the tubing with PTFE covered stents. In the circulation with uncovered stents the maximum expression appeared after 12 min for all measured antibodies (p = 0.05, Figs. 1, 2). In the control tubings the expression of CD62p and CD63 was increasing up to the end of measurement.

In vitro circulating model

First macroscopic visual platelet aggregates appeared after 5.6 ± 1.6 min in systems with PTFE-covered stents, whereas...
failed to demonstrate significant differences, they could only randomized study comparing covered and uncovered stents about restenosis and acute thrombotic occlusions after demonstrated promising results [11, 12]. Other authors reported about restenosis and acute thrombotic occlusions after PTFE-covered stents have the advantage of reducing peri-vascular restenosis. PTFE-coated guide-wire demonstrate a thrombus formation rate from 25 % to 69 % dependent on their design [16]. Thus, available data are still controversial. There are other in vitro models to test thrombogenicity of coronary stents that investigate coagulation factors, platelet beta-thromboglobulin, 11Indium labeled platelet accumulation, fibrinogen adsorption, and platelet adhesion. However, comparable in vitro data and test models are unfortunately not available. The present model does deliberately not apply whole blood. To study the interaction of platelets with e.g. leukocytes, monocytes or other blood corpuscles was not the target. Platelets and platelet activation are inherently not simple to detect by the technique of flow cytometry, standard deviations are substantial anyway. Thus, only platelet rich plasma was used to minimize additional artifacts induced by the other blood corpuscles. Our data revealed a time dependent activation of both platelets and the coagulation system. After detecting a maximum of epogetic antigen expression on platelets the number of measurable antigens decreased demonstrating a consumption of available platelets by aggregation, adhesion and thrombus formation in the tubing system. Thus, after 10 min in the tubings with PTFE-stents more measurements were not feasible. P-selectin and glycoprotein 53 are expressed in conditions of activated platelets, and seem to play a key role and appear to be most closely associated with an increase in thrombotic risk [17]. With PTFE-covered stents the expression of these glycoproteins was significantly higher as an evidence of additional platelet activation in contrast to the uncovered stents. In addition, thrombus formation was induced even by a higher degree by the teflon layer. In a recently published animal model, thrombus formation within the stent-graft interface was shown to promote neointimal development [18].

Other authors demonstrated a retarded neointimal hyperplasia only at the midportion of the devices, but did not prevent neointimal pannus ingrowth at the proximal and distal ends [14]. Thus, the question, whether an increased activation of platelets and the coagulation system may even enhance a neointimal covering of the stent can not be answered at this moment. However, in vitro studies are not necessarily reflecting clinical conditions especially the additional administration of clopidogrel and aspirin as currently recommended. They can only evaluate one fragment of a puzzle. This is a noteworthy limitation of this in vitro model. Increased activation of both platelets and coagulation system may or may not be of advantage in conjunction with stents-grafts. Although this in vitro model offers the opportunity to investigate in vitro and ex vivo impact of inhibitors of platelet aggregation and the coagulation cascade the purpose of this present study was exclusively to explore the genuine influence of PTFE on activation of platelets and coagulation. Additional investigations are needed to further evaluate the biological interactions and implications of PTFE-covered stents.

**Acknowledgements**

We would like to thank the company JOMED GmbH (Rangendingen, Germany) for supplying the investigated stents.

**References**


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