Thrombogenicity of Sirolimus-eluting Stents and Bare Metal Stents: Evaluation in the Early Phase after Stent Implantation

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Abstract. Background: Thrombogenicity of drug-eluting stents is a matter of controversial debate. The aim of this study was to evaluate the thrombogenicity of sirolimus-eluting stents (SES) compared to bare metal stents (BMS) in a standardised in vitro model. Materials and Methods: Nine SES and nine BMS were implanted in tubing loops and nine loops without stent served as controls. Initially and after 90 minutes of blood circulation in a modified chandler loop model, thrombin-antithrombin III complex (TAT), PMN-elastase, factor XIIa, SC5b-9, sP-selectin and platelet count were measured. Expression of CD62P, CD45/41 and PAC-1 on platelets were determined by flow cytometry. Results: After 90 minutes, platelet count decreased significantly in the loops with BMS and SES (p<0.05). Levels of TAT, PMN-elastase and SC5b-9 were significantly elevated after 90 minutes in all loops (p<0.05). sP-selectin significantly increased in the loops with BMS and SES after 90 minutes. No significant changes occurred in any flow cytometric data. Platelet count, sP-selectin, TAT, PMN-elastase, SC5b-9, CD62P, CD41/CD45 and PAC-1 showed no significant difference between BMS and SES. Conclusion: These data provide evidence that there is no difference in thrombogenicity of BMS and SES in the in vitro model.

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Materials and Methods

The experiments were performed by using a modified Chandler loop system as described previously (18). The rotating loops result in passive blood circulation to avoid possible cell damage and coagulation activation typical of an actively pumping system. The rotation speed was 15 rpm which resulted in a flow velocity of 12.5 cm/s. Heparin-coated loops were used (Carmeda; Medtronic, Netherlands) to minimise the coagulation activating effects of the tubing surface. The total length of the closed tubing was 50 cm. The temperature was maintained at 37°C by a water bath. Nine BMS (BX Velocity; Cordis, the Netherlands) and nine SES stents (Cypher; Cordis) were placed in polyvinyl chloride tubing loops. The total length of each stent was 23 mm with a diameter of 3.5 mm. Nine additional loops were kept plain to serve as controls.

Blood was obtained from nine volunteers (men, aged 22-35 years). All of them were apparently healthy non-smokers and claimed to not have taken any drugs in the 14 days before blood sampling. Blood was taken by venous puncture using 10 ml tubes. Heparin (Sarstedt, Germany) and 4.5 ml tubes (Becton & Dickinson, Belgium) containing CTAD. Similar samples were drawn from the Chandler loops after 90 minutes of circulation. Measurement of platelet count and flow cytometry were performed immediately. The tubes containing citrate were centrifuged at 3000 x g for 20 min. The separated plasma was then snap frozen and stored at −20°C until analysis.

Platelet count was assessed by a cell counter (Axon Lab AG, Switzerland), Quantitative detection of serum concentrations of sP-selectin was performed using an ELISA technique employing a commercially available assay kit (R&D Systems, Germany). Determination of thrombin-antithrombin III complex (TAT) was performed using an enzyme-linked immunosorbent assay (Enzygnost TAT micro; Dade Behring, Germany). For determination of PMN-elastase, an enzyme immunoassay (Merck, Germany) was used. SC5b-9 was also determined with an enzyme immunoassay (Quidel, USA). Determination of factor XIIa was performed using an ELISA-test in sandwich technique (Axis-Plant, Scotland).

To visualise microscopic changes on the stent surfaces after blood contact, electron microscopic scanning was performed. One BMS and one SES was left without blood contact for scanning electron microscopy and two BMS and two SES were scanned after 90 min of blood circulation.

Results

Soluble plasma markers. After 90 minutes, a trend towards lower platelet count was found in the loop without stent (Ctr). A significant decrease in platelet count was detected in the loop with BMS (p<0.05) and in the loop with SES (p<0.05) after 90 min of blood circulation, whereas no significant change was found between the two stent types. There was also a strong trend towards lower platelet counts in the loops with the stents compared with the control loop (Figure 1).

Significantly elevated TAT levels, PMN-elastase values and SC5b-9 levels were observed after 90 minutes of blood circulation in the control loop, the BMS and SES loop (p<0.05) compared to the initial values. The highest TAT level was seen in the BMS loop, while the highest SC5b-9 level was found in the SES loop. Between the loops, there was no significant difference concerning TAT, PMN-elastase, SC5b-9 and factor XIIa (Figure 1).

The assessed sP-selectin values after 90 min of blood circulation were significantly elevated in the BMS loop and the SES loop compared to the initial values. In the control loop, a trend towards elevated sP-selectin levels was found after 90 min. In both loops with stents, higher sP-selectin
values were detected compared with the control loop, although only the difference between the control loop and the BMS loops was significant ($p<0.05$). No significant difference of sP-selectin values were detected between the loops with BMS and SES (Figure 1).

Cellular adhesion molecules on platelets. The expression of cellular adhesion molecules on platelets after blood circulation in the Chandler loops is shown in Table I. The circulation of blood in the Chandler loop for 90 min resulted in no significant change in the expression of the cellular adhesion molecules CD62P and CD45/CD41 on platelets in all three loops. A trend towards a higher platelet expression of PAC-1 was found after 90 min compared to the beginning, but the results were not significantly different. The comparison of BMS with SES showed no significant differences regarding platelet expression of CD45/CD41, PAC-1 and CD62P after 90 min of blood circulation.

Scanning electron microscopy. Both stent types were covered with a polymer fibrin net and cellular deposition and multiple platelet aggregates after being exposed for 90 min to the circulating blood (Figure 2).

Discussion

Platelet and thrombin activation are important factors in the development of stent thrombosis (18). Patients with CHD often display an increased level of platelet activation even before coronary stenting (19). Increased activation of platelets is a consistent finding after coronary stent implantation (20). Combined therapy with aspirin and clopidogrel is recommended to inhibit platelet activity and prevent stent thrombosis. Extensive evaluations of thrombogenic properties of various stent types were performed by Tepe et al. (18). Beythien et al. (21) demonstrated that stent length has an effect on platelet activation. In the present experimental study,
Table I. Platelet surface expression of P-selectin (CD62P), platelet-leukocyte aggregates (CD41/45) and PAC-1 (CD62) at the beginning and after 90 min of blood circulation in the loops without a stent (Ctr), with a bare-metal stent (BMS) and with a sirolimus-eluting stent (SES). Data are presented as the means±standard error of mean.

<table>
<thead>
<tr>
<th></th>
<th>t = 0</th>
<th>Ctr</th>
<th>BMS</th>
<th>SES</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Selectin (CD62P) [%]</td>
<td>56.9±1.6</td>
<td>54.0±1.3</td>
<td>54.4±1.6</td>
<td>54.6±1.4</td>
</tr>
<tr>
<td>PAC-1 (CD62) [%]</td>
<td>22.5±2.4</td>
<td>33.9±6.9</td>
<td>35.9±8.2</td>
<td>28.5±5.7</td>
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<tr>
<td>CD41/CD45 [%]</td>
<td>77±7.1</td>
<td>76±2.4</td>
<td>77±2.2</td>
<td>77±2.0</td>
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BMS: bare-metal stents, Ctr.: control loop/loops without a stent, SES: sirolimus-eluting stents.

Flow cytometric analysis was used to demonstrate platelet activation and platelet-leukocyte interaction. In clinical trials, increased P-selectin surface expression predicts stent thrombosis (23). Coronary stent implantation with balloon-injury of the atherosclerotic plaque may lead to platelet activation due to intimal damage. In the in vitro system, without endothelial damage, no change in P-selectin expression, PAC-1 binding to activated GPIIb/IIIa receptors and platelet-leukocyte aggregates was detected. These data indicate that both stent types have similar actions on platelet activation and platelet-leukocyte interactions in vitro.

In conclusion, coronary stents are thrombogenic, and coating of the stents with sirolimus-impregnated polymers has no relevant effect on platelet binding and platelet activation in the early phase after stent placement. This emphasises the importance of combined antiplatelet therapy to be administered until complete endothelialisation of the stent surface has been achieved.

References


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