Reduction of monocyte–platelet interaction and monocyte activation in patients receiving antiplatelet therapy after coronary stent implantation

A. E. May, F.-J. Neumann, M. Gawaz, I. Ott, H. Walter and A. Schomig

1. Medizinische Klinik, Technische Universität München, München, Germany

Background Monocyte activation induces different procoagulant and proadhesive inflammatory responses and thus may play a role in thrombotic complications after coronary interventions. Monocyte–platelet interaction may trigger these effects inducing monocyte activation.

Aims To characterize the effect of antiplatelet vs anticoagulation therapy on monocyte–platelet interaction and monocyte function after intracoronary stenting.

Methods and Results Immediately before, and during the first 12 days after successful coronary stenting, monocyte–platelet conjugates and monocyte function were assessed by flow cytometric detection of GPIIb/IIIa (CD41) on monocytes and by monocyte surface exposure of Mac-1 (CD11b/CD18) and t-selectin (CD62L). Twenty patients receiving combined antiplatelet therapy (ticlopidine, aspirin) were compared to 20 patients with standard anticoagulation (phenprocoumon, overlapping heparin, aspirin). Before stenting, monocyte–platelet conjugates and Mac-1 surface expression in both groups were significantly increased, while t-selectin was significantly diminished. Anticoagulation did not change these variables significantly during the subsequent 12 days. In contrast, antiplatelet therapy reduced platelet–monocyte conjugates by 46 ± 9.3% (mean ± SEM, P=0.0019) within 4 days, which was associated with a decrease in Mac-1 expression (28 ± 6.7%, P=0.0013) and an increase in t-selectin (56 ± 15.0%, P=0.0061).

Conclusion After intracoronary stenting, combined antiplatelet therapy, but not anticoagulation, causes reduction of monocyte–platelet interaction, which is associated with monocyte deactivation. This may contribute to a decreased risk for thrombotic events.

Key Words: Stent, monocytes, ticlopidine, anticoagulation.

Introduction

Coronary stenting is an established treatment for suboptimal immediate results after angioplasty. Moreover, recent data suggest that in selected patients elective coronary stenting may improve long-term outcome. However, despite strict anticoagulation therapy, subacute stent thrombosis remains a major early complication. The activated platelet appears to play a central role in this event. Apart from their direct haemostatic effect, activated platelets may stimulate procoagulant inflammatory responses. Activated platelets are known to attach to monocytes and thereby to initiate monocyte activation. Activated monocytes may play a role in the pathogenesis of thrombosis via several pathways: they initiate the extrinsic pathway of coagulation via surface expression of tissue factor. Moreover, activated monocytes rapidly show enhanced surface expression of the β2-integrin Mac-1 (CD11b/CD18). Mac-1 binds and converts factor X to Xa, leading to rapid fibrin formation. Mac-1 also binds to intercellular adhesion molecule-1 (ICAM-1) on endothelial cells, facilitating tight adhesion as a prerequisite of monocyte transendothelial migration. In addition, activated monocytes release a variety of promotores of acute inflammatory response, such as metabolites of arachidonic acid, leukotrienes and interleukin-1.

In patients with coronary heart disease, increased monocyte surface expression of both the adhesion molecule Mac-1 and tissue factor were found. We have previously shown that after coronary stenting combined antiplatelet therapy with ticlopidine and aspirin reduces platelet activation, as compared with standard anticoagulation regimen using a vitamin K antagonist, overlapping heparin and aspirin.
Table 1 Criteria for increased risk for subacute stent thrombosis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Antiplatelet (n=20)</th>
<th>Antiplatelet (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stent as bailout for acute occlusion after coronary balloon angioplasty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stent in left anterior descending coronary artery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissection not fully covered by stent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombolysis in Myocardial Infarction grade 1 or 2 distal runoff after stenting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute myocardial infarction within the last two weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracoronary thrombus within stented segment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal lumen diameter within stent &lt;3 mm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Methods

Patients

Patients with successful intracoronary stent placement after PTCA, considered to be at low risk for subacute stent thrombosis, were eligible for the study. Indications for stenting were extensive coronary dissections after PTCA, complete vessel closure, residual stenosis of 30% or more, and lesions in venous bypass grafts. The risk of subacute stent thrombosis was stratified by a panel of angiographic and clinical risk factors and was considered low if the patients fulfilled less than two of the criteria shown in Table 1. We included 20 consecutive patients of a pilot study for the ISAR trial, treated by combined antiplatelet therapy, and 20 consecutive patients treated with anticoagulation who were recruited during the 3 months preceding this pilot study. The baseline clinical and angiographic characteristics of the study population are shown in Tables 2 and 3. The study was approved by our institutional ethics committee, and all patients gave written informed consent. In addition, 50 healthy volunteers (mean age 27 years; range 24-40 years) were recruited from the hospital staff to establish reference range values for cytometry.

Stent placement

Stent implantation was performed as previously described. Before PTCA, heparin 15 000 units and aspirin 500 mg were given intravenously. Conventional monorail balloon catheters were used for angioplasty (Express; Scimed, Verviers, Belgium). The 7 mm or the articulated 15 mm standard Palmaz-Schatz stent (Johnson & Johnson, Warren, NJ, U.S.A.) were hand-crimped on the angioplasty balloon. Balloon catheters

for stent deployment were chosen with the intention of slightly oversizing the balloon. In addition, non-compliant balloon catheters (High energy; Boston Scientific, Hilden, Germany) were used in most of the patients for higher pressure dilatation. If necessary, multiple stents were utilized for complete coverage of the dissection. As part of the stratification of the risk of subacute stent thrombosis, intravascular ultrasound was used routinely. Quantitative analyses of coronary angiograms were performed off-line on a digital angiographic work station (AWOS, Siemens, Munich, Germany). The arterial sheath was removed with a partial thromboplastin time below 60 s, typically within 3 h of the intervention. After manual compression of the groin, carried out as long as necessary for local haemostasis (at least 30 min), a pressure bandage was applied. Specific devices were not used.

Antiplatelet and anticoagulation regimen

Immediately after pressure bandage application, a continuous heparin infusion, titrated to a partial thromboplastin time of 80 to 100 s, was started in all patients. In patients receiving anticoagulation, the vitamin K antagonist phenprocoumon (Marcumar; Hoffmann-La Roche, Grenzach-Wyhlen, Germany) was initiated immediately after the intervention. Heparin infusion was continued for 5 to 10 days until a stable level of oral anticoagulation was achieved. The target International Normalized Ratio (INR) was between 3-5 and 4-5. Partial thromboplastin time and INR were monitored twice daily. In patients assigned to antiplatelet therapy, heparin was discontinued 12 h after the intervention. Ticlopidine 250 mg twice daily (Tiklyd, Sanofi-Winthrop, Munich, Germany) was started immediately after the procedure. Ticlopidine and phenprocoumon

None of the differences between the two groups were statistically significant (P>0.15).
were given for 4 weeks, and all patients in both groups received aspirin 100 mg twice daily continuously.

Flow cytometry

Blood samples were handled as described earlier. In brief, peripheral venous blood, anticoagulated with 1:5 (vol/vol) CPDA (sodium citrate, phosphate buffer, dextrose, adenine; Greiner, Germany), was obtained immediately before the intervention and thereafter daily in the morning for 12 days. Staining was performed immediately after blood sampling in whole blood. Whole blood (25 μl) and an equal volume of phosphate-buffered saline (pH 7.4) were incubated with saturating concentrations of phycoerythrin (PE)-conjugated anti-CD14 and with one of the fluorescein isothiocyanate (FITC)-labelled monoclonal antibodies (mAbs) for 30 min in the dark at room temperature. Erythrocytes were lysed and leukocytes were fixed (Lysing solution and fixing reagent, Coulter Electronics, Krefeld, Germany). Finally, the cells were washed three times and stored in paraformaldehyde (1%) at 4 °C in the dark. Flow cytometric analysis was performed within 24 h on a FACScan flow cytometer (Beckton Dickinson). The day-to-day reproducibility was controlled by fluorescent beads of defined various fluorescence (CaliBRITE, Becton Dickinson). Monocytes were identified by CD14-positive PE-fluorescence. In each sample, FITC-fluorescence intensity of 2000 CD14-positive cells was analysed over a logarithmic scale of 1 to 10^26 channels. Results are expressed as mean channel of fluorescence intensity (mean fl.).

Monoclonal antibodies

All mAbs were obtained from Immunotech. MAbs anti-CD11b (clone, Bear1), anti-CD62L: (clone, DREG56) and anti-CD41a (clone, P2) were obtained FITC-labelled. MAb anti-CD11b and anti-CD62L both served as markers of monocyte activation showing no cross-reaction with resting or activated platelets in control experiments. Activation of monocytes is typically accompanied by upregulation of the Mac-l-complex (CD11b/CD18) and shedding of L-selectin from the cellular surface. MAb anti-CD41 is directed against the glycoprotein IIb/IIIa, which is the inducible fibrinogen receptor, expressed on platelets. GPIIb/IIIa was analysed on the monocyte surface and served as a marker of platelet or platelet particle binding to monocytes. PE-conjugated mAb anti-CD14 (clone, TUK4) was used to identify monocytes. CD14 is a myeloid differentiation protein typically expressed on the surface of mature monocytes.

Statistical analysis

The Student’s t-test was used to test quantitative coronary angiography data. The Kolmogorov Smirnov test showed that the flow cytometry data were not normally distributed. The results are reported as median (interquartile range), unless otherwise indicated. The study primarily sought to test the hypothesis that immunofluorescence variables vary between the two treatment groups. Second, we analysed the time course of the immunofluorescence variables within each group.
Table 4  Monocyte surface markers before stent implantation

<table>
<thead>
<tr>
<th>Marker</th>
<th>Anticoagulation (n=20)</th>
<th>Antiplatelet (n=20)</th>
<th>P</th>
<th>Control (n=50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mac-1</td>
<td>107 [84;155]</td>
<td>128 [78;162]</td>
<td>0.68</td>
<td>71 [52;103]</td>
</tr>
<tr>
<td>L-selectin</td>
<td>30 [24;39]</td>
<td>38 [34;44]</td>
<td>0.11</td>
<td>62 [56;72]</td>
</tr>
<tr>
<td>GPIIb/IIIa</td>
<td>167 [74;260]</td>
<td>209 [142;352]</td>
<td>0.30</td>
<td>39 [34;85]</td>
</tr>
</tbody>
</table>

Mean channel fluorescence intensity is expressed as median [interquartile range]. P values refer to the differences between the two treatment groups. All markers in the control group showed a significant difference (P<0.01) to both patient groups.

Figure 1  (a) Monocyte surface exposure to GPIIb/IIIa (CD41) before stent implantation and during the first 12 days after intervention. Standard anticoagulation (n=20) (○) was compared to combined antiplatelet therapy (n=20) (●). *indicates significant (P<0.05) differences between both patient groups. □ indicates reference range. (b) Plot of the individual changes in the surface exposure of GPIIb/IIIa (CD41) on monocytes before stent implantation and at day 4 after the procedure.

Accordingly, we first tested differences between the two groups by comparing the sum of each measurement in a patient using the Mann–Whitney U-test. If this revealed significant differences, we compared the variable at the individual time points. To analyse time courses, differences were tested by Friedman’s test followed by the Wilcoxon rank sum test. A P value less than 0.05 was regarded as significant.

Results

Baseline characteristics of the study group

Twenty patients treated with anticoagulation and 20 patients receiving antiplatelet therapy were studied. As shown in Tables 2 and 3, the two patient groups were comparable with regard to their clinical and angiographical characteristics.

Before stent implantation, both patient groups showed significantly (P<0.01) increased monocyte immunofluorescence of Mac-1 and GPIIb/IIIa and significantly (P<0.01) decreased surface density of L-selectin compared to the reference range reflecting increased monocyte–platelet interaction and monocyte activation. Before stent implantation the three surface markers did not significantly differ between the two patient groups (Table 4).

Platelet–monocyte conjugates

In the anticoagulation group, GPIIb/IIIa on monocytes was constantly elevated for 12 days, whereas antiplatelet therapy caused a significant (P<0.01) persistent decrease in anti-GPIIb/IIIa immunofluorescence on monocytes after the intervention (day 4, 80[48–172] vs pre-stent, 209 [142–352], P<0.002) (Fig. 1). Significant differences between the two patient collectives were found on days 1, 2, 4, 5 and 8 after the intervention.
Monocyte surface markers

In patients receiving anticoagulation, Mac-1 surface expression remained elevated at the pre-stent level for the entire study period (Fig. 2(a)). In contrast, in the antiplatelet group Mac-1 decreased after stenting, reaching statistical significance vs pre-stent values at day 4 (mean channel fluorescence intensity, median [interquartile range]; day 4, 77 [60–106] vs pre-stent, 128 [78–162], P<0.002) (Fig. 2(b)). Thereafter, Mac-1 essentially remained at the same level during the whole study period. Significant differences between the two patient groups were found on days 4, 5, 9, 10 and 11.

In patients with anticoagulation, L-selectin surface expression essentially remained constant at the low peri-interventional level. In contrast, in the antiplatelet group, L-selectin on monocytes increased within 4 days up to the level of the healthy control group (mean channel fluorescence intensity, median [interquartile range]; day 4, 66 [43–81] vs pre-stent, 36 [31–48], P<0.01) and remained constant thereafter (Fig. 3). Significant differences between the two patient groups were found on days 1–4 after the intervention.

Discussion

This study compares antiplatelet with anticoagulation therapy after coronary stenting and their effect on platelet–monocyte interaction and monocyte function during the first 12 days after intervention. In both groups, platelet–monocyte conjugates were substantially above the reference range at pre-stent, and the surface expression of Mac-1 and L-selectin indicated monocyte activation. In patients receiving combined antiplatelet therapy, we found normalization of platelet–monocyte conjugates after stenting, which was associated with monocyte deactivation. Anticoagulation therapy, however, did not affect platelet–monocyte conjugates and concomitantly the level of monocyte activation assessed by surface markers remained elevated throughout the observation period.

Platelet–monocyte conjugates

Platelets adhere to leukocytes in an activation-dependent manner and modulate the activation state of leukocytes. Specifically, we showed that adherence of monocytes to platelets induces the expression of interleukin-1β, interleukin-8 and MCP-1. Previous studies showed platelet activation in patients with advanced coronary artery disease and demonstrated that platelet function was predictive for the risk of acute ischaemic events after coronary interventions. In accordance with our previous study, we found elevated levels of platelet–monocyte interaction before coronary stenting. This finding may be explained as a consequence of platelet activation due to high shear stresses at narrow coronary artery stenoses. After coronary stenting, combined antiplatelet therapy decreases platelet activation. Consistent with these findings, the present study shows decreased platelet–monocyte interaction in patients treated with antiplatelet therapy.
Monocyte activation

Platelet–leukocyte interaction is associated with leukocyte activation. It induces upregulation of Mac-1, proteolytic shedding of L-selectin, superoxide anion release and increased tissue factor expression. In our patients, platelet–monocyte interaction paralleled the increased surface expression of Mac-1 and shedding of L-selectin. Both platelet–monocyte conjugates and monocyte activation markers were lowered by combined antiplatelet therapy after the intervention. The surface markers of monocyte activation investigated in this study are of major physiological importance: they promote local inflammation and thrombogenesis by mediating intercellular adhesion and induction of procoagulant activity; whereas L-selectin initiates the primary contact (rolling) of monocytes onto endothelium, Mac-1 mediates tight monocyte-endothelial adhesion (arrest). Moreover, occupancy of the Mac-1 receptor sets the signal for increased surface expression of tissue factor. In addition, Mac-1 can bind and convert factor X to factor Xa. Therefore, activation-dependent modulation of these two surface molecules may directly influence inflammatory and procoagulant processes such as subacute stent thrombosis.

Limitations of the study

Assignment to anticoagulation or antiplatelet therapy was not randomized in our study. Hence, there is concern that the results of this study might have been affected by differences in clinical angiographic characteristics or changes in the stenting procedure. However, the inclusion of patients with low risk of stent thrombosis only, ensured that the study population was quite homogeneous and our procedural approach was not changed during the study period. Accordingly, the two study groups did not differ with respect to any of the relevant procedural, angiographic or clinical characteristics. Therefore, we do not have sound reason to assume that the results of our study would have been substantially different, if assignment to treatment had been randomized.

Clinical implications

Our study demonstrates that, compared with conventional anticoagulation, combined antiplatelet therapy after coronary stenting may exert anti-inflammatory effects.
Reduction of monocyte–platelet interaction and monocyte activation

Effects by decreased surface expression of Mac-1. Put together with the results of previous in vitro studies12,20,31 our findings suggest that this effect is mediated through reduction of monocyte activation by adherent activated platelets. It is known that inhibition of monocyte activation not only affects the surface expression of adhesion molecules and procoagulant activity, but also the secretion of cytokines30. Monocyte-derived cytokines such as interleukin-1β and tumour necrosis factor-α play an important role in the initiation of the systemic inflammatory response syndrome39 commonly found in acute coronary syndromes22. Among the sequelae of these inflammatory responses, the increase in plasma fibrinogen is a notorious cardiovascular risk factor40, so far difficult to treat. Ticlopidine administration has already been shown to reduce plasma fibrinogen levels41. The potential indirect anti-inflammatory effects of antplatelet vs anticoagulation therapy on monocyte function may contribute to a decreased risk for subacute stent thrombosis and increased survival in patients receiving antplatelet therapy after intracoronary stenting, as found in the ISAR trial22. If the findings of the present study can be extrapolated to other manifestations of ischaemic heart disease, potent inhibition of platelet function may be considered as a potential means of inhibiting detrimental systemic inflammatory responses in acute coronary syndromes.

The authors thank Kathrin Schulz and Corinna Kraft for excellent technical assistance.

References


