Catheter based intracoronary brachytherapy leads to increased platelet activation

M Jaster, V Fuster, P Rosenthal, M Pauschinger, Q-V Tran, D Janssen, W Hinkelbein, P Schwimmbeck, H-P Schultheiss, U Rauch

Background: Vascular brachytherapy (VBT) after percutaneous coronary intervention (PCI) is associated with a higher risk of stent thrombosis than conventional treatment.

Objective: To investigate in vivo periprocedural platelet activation with and without VBT, and to assess a possible direct effect of radiation on platelet activation.

Design: Of 50 patients with stable angina, 23 received VBT after PCI, while 27 had PCI only. The 23 patients who received VBT after PCI were pretreated for one month with aspirin and clopidogrel. Platelet activation was assessed by flow cytometry.

Results: The two patient groups did not differ in their platelet activation before the intervention. There was a significant increase in activation immediately after VBT, with 23.7% (interquartile range 13.0% to 37.6%) thrombospondin positive and 54.0% (42.3% to 63.6%) CD 63 positive platelets compared with 12.7% (9.8% to 14.9%) thrombospondin positive and 37.9% (33.2% to 45.2%) CD 63 positive platelets before the intervention (p < 0.001 and p < 0.01, respectively). Patients without VBT had no periprocedural difference in platelet activation immediately after PCI. No increase in platelet activation was found after ex vivo irradiation of blood samples obtained from healthy controls.

Conclusions: Catheter based intracoronary VBT carried out according to current standards is highly thrombogenic. The current anti-thrombotic treatment with aspirin and clopidogrel is not sufficient to suppress platelet activation during the procedure. From in vitro experiments, it appears that platelet activation during brachytherapy is not caused by irradiation but by the procedure of catheter based VBT.

Methods

Patients

The study included 50 patients with stable angina, 23 of whom (mean (SD) age, 61 (9) years; 19 men, four women) were treated with the Novoste Beta-Cath system for in-stent restenosis after successful primary intervention. Twenty seven patients (58 (9) years; 23 men, four women) underwent PCI without irradiation served as controls. Brachytherapy and control patients did not differ significantly in age, sex, distribution of coronary risk factors, and number of diseased vessels (table 1). Non-ionic contrast medium was used in all patients (Ultravis 370, Schering, Berlin, Germany). The left circumflex artery was treated more often in controls.

The radioactive source used was 90Sr/90Y, a β emitter with a maximum energy of 2.27 MeV. All irradiated patients were treated with the same source trains between March and October 2000 after informed written consent and approval by the local ethics committee. Radiation doses were 18.4, 23.0, or 25.3 Gy, depending on the major vessel diameter accessed by intravascular ultrasound (IVUS) (table 2). We used two different source trains: one device 40 mm long with 16 active encapsulated seeds and the other 60 mm long with 24 seeds.

Abbreviations: FITC, fluorescein isothiocyanate; IVUS, intravascular ultrasound; PCI, percutaneous coronary intervention; TSP, thrombospondin; VBT, vascular brachytherapy
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was done to confirm a residual stenosis with a lumen
dure was applied at 1 mm/s. After balloon angioplasty, IVUS
visualised in-stent restenosis. A motorised pull back proce-
sion was excluded as the cause of the angiographically
50% cross sectional area. Stent malposition or underexpan-
confirm a significant in-stent restenosis—that is, more than
(Endosonics) was carried out in irradiated patients to
clotting time (ACT) of 300 seconds. Intravascular ultrasound
placement was undertaken during brachytherapy.

The dose rate at the target point 2 mm away from the centre of
the source train was determined to be 0.1VGy/s for the 40 mm
source train and 0.08 Gy/s for the 60 mm source train, with a
longer radiation time to achieve an equal dose. No new stent
placement was undertaken during brachytherapy.

All patients treated with brachytherapy received dual
antiplatelet treatment with aspirin and an ADP receptor
antagonist (clopidogrel 300 mg loading dose followed by
75 mg per day) three to four weeks before and at least six
months after elective irradiation. No pretreatment was given
in the control group. Control patients were treated with
aspirin and additionally received an ADP receptor antagonist
for two weeks after the intervention only in cases of stent
implantation (12/27) or stent angioplasty (5/27). No glyco-
protein IIb/IIIa receptor antagonist was used in any of the
cases.

Blood samples were taken before and immediately after
the intervention in the catheter laboratory, during effective
heparinisation and before the removal of the venous sheath
in all patients. The blood was analysed immediately. Blood
samples after 24 hours were taken the next morning before
breakfast.

IVUS
Intravenous heparin was given to obtain a target activated
clotting time (ACT) of 300 seconds. Intravascular ultrasound
(Endosonics) was carried out in irradiated patients to
confirm a significant in-stent restenosis—that is, more than
50% cross sectional area. Stent malposition or underexpansion
was excluded as the cause of the angiographically
visualised in-stent restenosis. A motorised pull back proce-
dure was applied at 1 mm/s. After balloon angioplasty, IVUS
was done to confirm a residual stenosis with a lumen
diameter of less than 30%. The elastica externa was assessed
as the vessel diameter containing the target cells for
irradiation.

Platelet activation measurement
Whole blood was collected in a special sample medium
(containing EDTA, hydroxychloroquine sulfate, sodium
hydroxide, and heparin sodium) through a 1 mm
Wassermann cannula without a tourniquet and immediately
fixed in paraformaldehyde (1%). Platelet activation was
determined by flow cytometry as described. In short,
platelet-rich plasma was obtained by centrifugation at 100 g
for 10 minutes (room temperature). Platelets were counted
and diluted with phosphate buffered saline to a final
concentration of 1000/nl. Aliquots were incubated with
saturated concentrations of monoclonal antibodies against
the activation dependent activation markers CD 63 and
thrombospondin and the constitutively expressed glycopro-
tein IIb/IIIa. A second FITC labelled anti-mouse antibody was
added to detect the primary antibodies. Flow cytometry was
then carried out, with 10 000 events acquired for each
sample. Data are given as the percentage of marker positive
platelets for thrombospondin and CD 63 or as the mean
immunofluorescence intensity for glycoprotein IIb/IIIa.

Ex vivo radiation experiments
Blood samples were obtained from healthy subjects without
a tourniquet and immediately mixed with citrate. Whole blood
was divided into aliquots, one serving as a control and the
others being irradiated. Two different doses (20 Gy and
160 Gy) were used in repeated experiments to cover the dose
range applied to blood in patients undergoing brachytherapy.
A linear accelerator with a 6 or 18 MeV photon beam served
as the radiation source. Immediately after irradiation, which
took about 15 minutes, all aliquots were fixed in 1%
paraformaldehyde and analysed by flow cytometry as above.

Statistics
If data were normally distributed, Student’s t test was used to
compare continuous variables. Results are expressed as mean
(SD). If data were not normally distributed, we used
Wilcoxon’s rank test to compare continuous variables.
Results are expressed as medians and interquartile ranges.
Binary variables were compared using the χ² test or Fisher’s
exact test when appropriate. The Mann-Whitney U test was
chosen to compare different groups. A probability value of
p < 0.05 in the two sided test was considered significant.

Table 1 Clinical details of the patients and controls

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Patients treated with brachytherapy (n = 23)</th>
<th>Patients treated without irradiation (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>61 (9)</td>
<td>58 (9)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>4/19</td>
<td>4/23</td>
</tr>
<tr>
<td>Treated coronary artery</td>
<td>12/23</td>
<td>11/27</td>
</tr>
<tr>
<td>LAD</td>
<td>1/23</td>
<td>9/27</td>
</tr>
<tr>
<td>LCx</td>
<td>2/23</td>
<td>15/27</td>
</tr>
<tr>
<td>RCA</td>
<td>10/23</td>
<td>7/27</td>
</tr>
<tr>
<td>Number of diseased arteries</td>
<td>1.9 (0.8)</td>
<td>2.2 (0.7)</td>
</tr>
<tr>
<td>Smoking</td>
<td>14/23</td>
<td>16/27</td>
</tr>
<tr>
<td>Diabetes</td>
<td>5/23</td>
<td>7/27</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1/23</td>
<td>13/27</td>
</tr>
<tr>
<td>Lipids</td>
<td>20/23</td>
<td>22/27</td>
</tr>
<tr>
<td>Family history</td>
<td>1/23</td>
<td>2/27</td>
</tr>
<tr>
<td>ACT of the procedure (s)</td>
<td>359 (64)</td>
<td>342 (67)</td>
</tr>
</tbody>
</table>

Values are mean (SD) or n. *p<0.05
ACT, activated clotting time; LAD, left anterior descending coronary artery; LCx, left circumflex artery; RCA, right coronary artery.

Table 2 Comparison of the different source trains used in our study between March and September 2000

<table>
<thead>
<tr>
<th>40 mm source train</th>
<th>60 mm source train</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients treated with different train doses (n)</td>
<td></td>
</tr>
<tr>
<td>18.4 Gy</td>
<td>19/23</td>
</tr>
<tr>
<td>23.0 Gy</td>
<td>7/19</td>
</tr>
<tr>
<td>25.3 Gy</td>
<td>6/19</td>
</tr>
</tbody>
</table>

The dose rate varies for each source train and depends on the time of radioactivity storage.
Platelet activation in patients treated with and without vascular brachytherapy

<table>
<thead>
<tr>
<th></th>
<th>Treated with VBT (n = 23)</th>
<th>Controls (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% TSP positive platelets in blood before the intervention</td>
<td>12.7 (9.8 to 14.9)</td>
<td>10.9 (8.8 to 14.1)</td>
</tr>
<tr>
<td>% TSP positive platelets in blood after the intervention</td>
<td>21.2 (13.0 to 37.6)</td>
<td>9.2 (7.4 to 10.1)</td>
</tr>
<tr>
<td>% CD 63 positive platelets in blood before the intervention</td>
<td>37.9 (33.2 to 45.2)</td>
<td>46.3 (33.7 to 51.3)</td>
</tr>
<tr>
<td>% CD 63 positive platelets in blood after the intervention</td>
<td>54.0 (42.3 to 63.6)</td>
<td>41.2 (34.1 to 49.8)</td>
</tr>
<tr>
<td>GP IIb/IIIa on platelet surface before the intervention</td>
<td>305.7 (269.5 to 381.2)</td>
<td>309.3 (276.9 to 365.4)</td>
</tr>
<tr>
<td>GP IIb/IIIa on platelet surface after the intervention</td>
<td>299.7 (263.1 to 377.4)</td>
<td>301.0 (261.7 to 384.7)</td>
</tr>
</tbody>
</table>

Platelet activation is indicated by the median and interquartile range of TSP positive and CD 63 positive platelets before and after the intervention. *p < 0.01 between patient groups (Mann-Whitney U test); †p < 0.001 between patient groups; ‡p < 0.01 pre v post intervention in patients treated with VBT (Wilcoxon’s rank test); glycoprotein IIb/IIIa remained unchanged (NS).

GP, glycoprotein; TSP, thrombospondin; VBT, vascular brachytherapy.

### RESULTS

The two patient groups were comparable in their clinical data (table 1). Table 2 shows the number of patients treated with different source trains. Before the intervention, the two patient groups did not differ significantly with respect to the in vivo activation of circulating platelets (table 3). There was a significant increase in thrombospondin positive platelets immediately after PCI with brachytherapy compared with the percentage found before the intervention (p < 0.01) or after PCI without irradiation (p < 0.001) (fig 1A). CD 63 positive platelets also increased in VBT treated patients immediately after the intervention compared with the CD 63 before the intervention (p < 0.01) or after PCI with irradiation (p < 0.01) (table 3). These data indicate that catheter based intracoronary brachytherapy is associated with increased in vivo platelet reactivity. Glycoprotein IIb/IIIa on the platelet surface remained unchanged and was comparable in the two patient groups. There was no heterogeneity in the actual sampling time between the groups.

IVUS was used before and after intervention in the VBT group. IVUS itself may cause plaque perturbation in the coronary artery, leading to increased platelet reactivity. To rule this out as a possible effect, platelet activation was assessed in a small control group (n = 6), in which IVUS was done before and after stent implantation. The percentage of TSP and CD 63 positive platelets was not altered during intervention (TSP: 10.9% (interquartile range 8.3% to 18.2%) before 12.0% (9.4% to 14.5%) immediately after intervention, p = 0.917; for CD 63: 32.4% (30.6% to 46.0%) before v 36.2% (33.7% to 42.4%) immediately after intervention, p = 0.753).

### In vitro experiments

Irradiation of blood samples (not platelet-rich plasma) with target doses used in humans did not affect platelet activation. Even eight times higher doses did not increase platelet activation compared with the non-irradiated control aliquots (table 4). This indicates that platelet activation is not directly affected by irradiation.

### DISCUSSION

This study is the first to show that platelet activation is increased by the brachytherapy currently applied in clinical practice. A notable increase was found after brachytherapy compared with PCI without irradiation. We have previously identified increased platelet activation after conventional PCI as an independent risk factor for acute ischaemic events. The data on radiation delivery devices submitted for US Food and Drug Administration (FDA) approval showed that patients treated with radiation benefit from a reduction in recurrent stenosis but also have the disadvantage of an increased rate of thrombotic events. Thrombotic occlusions are associated with increased mortality after PCI and an increased risk of myocardial infarctions after...
The increase in platelet activation after brachytherapy is not suppressed by concomitant treatment with aspirin and clopidogrel. Both drugs were given to all 23 patients three to four weeks before elective brachytherapy. In the group of control patients, only those with PCI for in-stent restenosis or new stent implantation received ADP receptor antagonists in addition to aspirin. In these control patients, treatment with an ADP receptor antagonist started during the intervention. Control patients showed no change in platelet activation after the intervention. The results coincide with previous reports on periprocedural platelet activation in stable angina patients treated with PCI.27

The differences in platelet activation after the intervention. The results coincide with previous reports on periprocedural platelet activation in stable angina patients treated with PCI.27 The differences in medical treatment explain the tendency towards fewer CD 63 positive platelets before the intervention in patients who underwent VBT after taking antiplatelet treatment for three to four weeks. The lysosomal glycoprotein CD 63 is a marker for prolonged and severe platelet activation. Despite pretreatment with aspirin and clopidogrel for three to four weeks, platelet activation—as found for TSP and CD 63—increased during intracoronary brachytherapy. The more intense pre-treatment with thienopyridines in the brachytherapy group makes the results even more striking.

Our results support the discussion of three different mechanisms for increased platelet reactivity after VBT: first, the irradiation itself affects in vivo platelet activation and blood thrombogenicity; second, the currently used catheter based radiation source triggers and their time consuming procedures may be highly thromogenic; and third, VBT affects platelet activation indirectly owing to endothelial damage. Nitric oxide/endothelium derived relaxing factor (NO/EDRF) produced by the intact endothelium inhibits platelet activation. This endothelium derived antithrombogenic effect may be reduced after VBT.

We undertook in vitro experiments to rule out a direct effect of irradiation on platelet activation. The doses used were biologically equivalent to those delivered by the radioactive sources. There was no direct radiation dependent effect on platelet activation in these in vitro experiments (table 4). The findings coincide with previous reports on short term irradiation in doses of up to 150 Gy.30 It remains to be shown whether the degree of platelet activation is determined by the procedure of catheter based intracoronary brachytherapy or by the effects of VBT on endothelial function. Platelet activation may be influenced by the length of the source passage. Patients treated with the endovascular device and the indwelling time of the highly thrombogenic catheter based delivery system in the coronary artery. However, it can also be hypothesised that the negative impact of VBT on endothelial function may affect platelet reactivity after intracoronary brachytherapy.

Recent reports on VBT in patients receiving combined antiplatelet treatment (aspirin plus ADP receptor antagonist) showed an increased rate of subacute thrombosis after PCI with irradiation31 32 compared with stenting without VBT.29 30 Our data showed that platelet activation after intracoronary brachytherapy was only incompletely suppressed by antiplatelet treatment with aspirin and clopidogrel. The second blood samples were taken immediately after the intervention during effective heparinisation. A possible effect of heparinisation on platelet activation cannot be excluded. However, all patients received heparin, so the groups remain comparable and the increase in platelet activation was found in the VBT group only.

Increased platelet reactivity after brachytherapy may trigger acute and subacute stent thrombosis after VBT and increase the number of myocardial infarcts, especially after new stent placement during VBT.21 22 In an analysis of 473 patients with in-stent restenosis enrolled in various VBT protocols, subacute thrombosis occurred in three patients (0.9%) treated with VBT and in none of the placebo treated patients.35 The acute changes in platelet activation observed here cannot be related to alterations in platelet reactivity or thrombotic events found three to six months after VBT (late thrombosis). Factors to be considered in connection with late thrombosis are the damaged endothelium and thrombogenic vasculature at the site of injury where re-endothelialisation is delayed or fails to occur after irradiation.33 34

Conclusions

Platelet activation is increased after catheter based VBT compared with PCI without irradiation. This increase occurs despite aggressive antiplatelet treatment with aspirin and clopidogrel given for three to four weeks before brachytherapy. Our in vitro experiments did not show a direct effect of irradiation on platelets and indicated that catheter based VBT involving endothelial damage is a highly thrombogenic procedure.

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REFERENCES
Ruptured plaque or embolised thrombus

A 38 year old woman with acute chest pain was admitted to the hospital. Positive troponins and electrocardiographic signs of anterior wall myocardial infarction during atrial fibrillation were found. Echocardiography (panel below) showed hypokinesia of the anterior wall and severe mitral stenosis (arrowhead; LV, left ventricle; SC, spontaneous contrast). Coronary angiography showed an occlusion of the mid left anterior descending artery (LAD) (panel A, upper row). Using the Guardwire Plus system (Medtronic Inc), the wire was positioned in the distal LAD and three aspirations during distal balloon inflation were performed resulting in complete reperfusion (TIMI-III flow) (panel B, upper row) of an angiographically normal appearing LAD (arrow) and normalisation of ST-T segments. The retrieved debris, a large irregular shaped particle (panel A, lower row, arrow), was histopathologically analysed and showed predominantly thrombus (T) with small but non-atherosclerotic parts of vascular media (M) and intima (I) (panel B, lower row, Mason’s trichrome). An additional stain for inflammation showed that CD-68 positive macrophages within this part of the aspirate were most likely obtained following traumatic passage of the aspiration catheter. The patient afterwards underwent mitral valve surgery as well as removal of the intra-atrial thrombus, and recovered well.

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Images in Cardiology