Increased Plasma Beta-Thromboglobulin in Patients With Coronary Artery Vein Graft Occlusion: Response to Low Dose Aspirin

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The therapeutic effect of aspirin on vein graft patency was studied in patients undergoing coronary artery bypass graft surgery. The study design enabled the prospective evaluation of the relation of platelet activation, as measured by plasma beta-thromboglobulin concentration, to subsequent coronary vein graft occlusion. Serial beta-thromboglobulin levels were measured in 105 patients randomized to receive aspirin (324 mg/day) or placebo beginning within 1 h after surgery. Graft patency was assessed angiographically at 1 week and 1 year after surgery.

Of 49 patients receiving placebo, 17 (34.7%) had one or more graft occlusions, 6 early, 10 late and 1 with both early and late occlusion. Of 56 patients receiving aspirin, 7 (12.5%) had one or more occlusions, 3 early and 4 late (p < 0.01). Preoperatively, the beta-thromboglobulin level in surgical patients (29 ± 13.5 ng/ml) was significantly higher than that of 51 control subjects (22.6 ± 11.1 ng/ml) (p < 0.004).

Plasma beta-thromboglobulin levels remained comparatively constant at 3 and 12 months after surgery in the 43 patients who had both samples available (p < 0.001, r = 0.65). The reduction in beta-thromboglobulin concentration from the preoperative level to 12 months postoperatively was greater in the aspirin-treated group (p < 0.001). Multivariate logistic regression analysis demonstrated a significant association between preoperative beta-thromboglobulin concentration and graft occlusion (p < 0.02), and aspirin treatment was effective in preventing occlusion when adjusted for the preoperative beta-thromboglobulin level (p < 0.005).

Plasma beta-thromboglobulin concentrations are elevated in patients with coronary artery disease, suggesting ongoing platelet activation. The risk of coronary artery bypass graft occlusion is greater in patients with higher preoperative levels of beta-thromboglobulin, a risk that is greatly reduced by aspirin therapy begun within 1 h after surgery and continued for 12 months postoperatively.

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tients with unstable angina (21) and delaying the progression of peripheral arterial disease (22).

The occlusion of saphenous vein bypass grafts within the first postoperative year is predominantly a result of thrombosis and diffuse intimal hyperplasia. Both these processes appear to be platelet-mediated (23). Although elevated beta-thromboglobulin may be regarded as a nonspecific index of platelet activation, this prospective study was undertaken to show whether there is a significant relation between preoperative plasma beta-thromboglobulin concentration and the occlusion of saphenous vein bypass grafts in a randomized placebo-controlled trial of aspirin therapy after coronary artery bypass surgery. A relation between anticardiolipin antibodies and graft occlusion was previously reported in a preliminary analysis of the first 83 patients studied (24).

Methods

Study patients. From August 1984 through October 1985, 383 patients undergoing primary aortocoronary saphenous vein bypass grafting at St. Vincent’s Hospital were screened for entry into a prospective, randomized, double-blind, placebo-controlled trial of aspirin for prevention of graft occlusion during the first postoperative year; 138 patients (36%) were considered eligible under the protocol and 137 patients consented to participate in the study. Criteria for exclusion from the study included age >70 years (n = 35), use of platelet inhibiting or antiinflammatory drugs within 7 days before surgery (n = 70), contraindications to aspirin therapy (n = 42), other vascular disease likely to require antithrombotic treatment (n = 35), presence of insulin-dependent diabetes mellitus (n = 19), coexistent connective tissue or other autoimmune disease (n = 5), nonfluency in English preventing an adequate explanation of need for follow-up angiography and tablet compliance (n = 3) and miscellaneous factors including history of noncompliance, significant renal impairment (creatinine >0.15 mmol/liter), isolated internal mammary artery grafting, severe peripheral vascular disease and severe left ventricular dysfunction with ejection fraction <20% precluding safe repeat angiography (n = 37).

Study protocol. After informed consent was obtained before surgery, patients were assigned to therapy in a randomized double-blind fashion. The randomization scheme involved a series of computer-generated random numbers, and patients were randomized by the dispensing pharmacist and stratified by each of five surgeons. Of the 137 patients entered into the study, 68 were assigned to aspirin therapy and 69 to placebo. The protocol conformed to the National Health and Medical Research Council guidelines for human experimentation and was approved by the Ethics and Research Committee of St. Vincent’s Hospital.

The study medication consisted of soluble aspirin (324 mg) or a matching placebo tablet to be commenced 1 h after leaving the operating theater and daily thereafter. The medication was dissolved in 30 ml of water and given by nasogastric tube (90 min clamp time) and repeated daily until the patient could tolerate oral intake.

Surgery. Cardiopulmonary bypass was established and myocardial preservation was effected by means of multiple dose cold blood cardioplegia (5°C to 7°C) and systemic cooling to 24° to 27°C. Saphenous veins were carefully resected, gently distended with heparinized whole blood and stored in blood until implantation. Distal anastomoses were performed with 7-0 and proximal anastomoses with 5-0 Prolene sutures during rewarming. Grafts were constructed as single grafts, sequential grafts or occasionally branched Y grafts. Coronary endarterectomy was performed in diffusely diseased distal arteries at the discretion of the surgeon. Patients were given heparin at the commencement of cardiopulmonary bypass, and this was promptly reversed with protamine sulfate before removal of the aortic cannula. Blood loss and transfusion requirements were recorded for both treatment groups.

Beta-thromboglobulin studies. Venous blood (4.5 ml) was drawn through a 21 gauge scalp vein needle, avoiding stasis and after the first 3 ml was discarded. The blood was mixed with 0.5 ml of an ethylenediaminetetraacetic acid (EDTA)-theophylline-prostaglandin E1 mixture (25) in a precooled tube, placed directly on ice and then centrifuged within 30 min at 4°C in a “Hettich” Mikroliter centrifuge (model 2020) at 8,000 g for 5 min. The top half of the plasma column was transferred to another precooled tube and centrifuged at 8,000 g for a further 5 min. The upper 1.5 ml of plasma was aspirated and stored at −70°C for assay. Radioimmunoassay of beta-thromboglobulin was carried out using a solid phase system in microtiter wells with a “sandwich” of goat anti-rabbit immunoglobulin (Ig) (F2-specific), rabbit anti-beta-thromboglobulin serum and indium-125-labeled beta-thromboglobulin. The assay was calibrated with World Health Organization 1st International standard beta-thromboglobulin, and all assays were carried out using both undiluted and a 1:2 dilution of plasma to ensure parallelism. All plasma samples from each individual patient were processed in one assay to avoid error from interassay variation. The within assay and between assay coefficient of variation was 6.8% and 10.7%, respectively, calculated from duplicate standards of 25 IU of beta-thromboglobulin included in each of seven assays.

Plasma beta-thromboglobulin concentrations >100 ng/ml were regarded as likely to be affected by artifactual in vitro platelet granule release and were excluded before analysis. There were six such assay results: two A samples, 127 and 718 ng/ml, respectively; two B samples, 234 and 419 ng/ml, respectively; and two D samples, 117 and 515 ng/ml, respectively.

Of the 137 patients entered into the study, 105 had at least two serial blood samples for beta-thromboglobulin assay. In
the remaining 32 patients, only a single preoperative sample or no sample was collected as a result of logistic problems with preparation of samples or patient refusal. Blood samples were taken 2 days preoperatively, before routine laboratory screening and ≥7 days after invasive diagnostic procedures (sample A); between 6 and 60 days (median 7) postoperatively, immediately before postoperative graft angiography (sample B); approximately 3 months postoperatively at follow-up visit (sample C); and between 222 and 420 days (median 363) postoperatively, before follow-up graft angiography (sample D). All blood samples were collected and prepared for assay by the same physician (T.P.G.).

D-dimer studies. The level of D-dimer in 96 citrated plasma samples collected preoperatively was assayed by the Dimertest-EIA (Mabco), which uses a monoclonal antibody specific for the D-dimer plasmin degradation product of human cross-linked fibrin (26).

Control group. Control samples were obtained in identical fashion from 51 healthy male and female hospital employees throughout the course of the study. These control subjects had not ingested aspirin or other antiplatelet agents within 7 days before venipuncture and had no known history or symptoms of cardiovascular disease or any hematologic disorder.

Graft angiography. Vein graft angiography was performed a median of 7 days (range 6 to 60) after operation in all 105 patients with adequate blood samples. The transfemoral Judkins technique was employed in all cases. The late vein graft angiogram was performed in 98 patients a median of 363 days (range 222 to 420) after surgery. There was no difference in the time to early or late angiography between the aspirin and placebo groups.

Vein grafts were selectively injected in orthogonal planes with multiple projections, and angiograms were analyzed by two independent, blinded observers. A third observer reviewed the films in cases of disagreement. If the aortic origin of the graft could not be visualized selectively, biplanar aortic root angiography was performed. Criteria for graft occlusion were those previously reported (16). A crude assessment of graft narrowing (diffuse or discrete) was made from orthogonal spot films of single vein grafts, the caliber at 1 year being compared with that at 1 week in similar projection planes. The degree of diffuse narrowing was recorded as mild (<30%), moderate (31% to 70%) or severe (71% to 99%). The interobserver variability for graft patency assessed by graft angiography was 4.5%. There was less agreement, however, in the assessment of stenosis of individual grafts, with a variability of 10.3%. The results reported reflect the consensus opinion of two of the three observers.

Compliance and follow-up. Patients were given a 4 month supply of the study medication (provided by Bayer Australia), with tablet record diaries to encourage compliance. At the first of three follow-up visits, a mid-morning urine sample was collected and stored at -20°C for a qualitative test for salicyluric acid (performed at the end of the study, using 5% ferric chloride solution to detect aspirin ingested within the past 12 h) and pill counts were performed. Patients were given paracetamol as an analgesic to discourage inadvertent use of aspirin-containing compounds. Platelet aggregation studies (using adrenalin, adenosine diphosphate and collagen) were performed for every sixth patient entered into the trial to further validate compliance.

Clinical data. The baseline clinical characteristics were obtained by interview with the principal investigator, with specific details of previous myocardial infarction and anginal history being recorded from inpatient records, preoperative cardiac catheterization, electrocardiography at rest and letters from the referring physician. Ongoing data collection concerning recurrent angina or other thrombotic events during the first postoperative year was also carried out by the principal investigator who did not know the patients allocated study medication.

Data analysis. Data were collated with use of Datastar (Micropro). Unless stated otherwise, values are expressed as mean values ± SD. Proportions of placebo and treatment groups were compared with Fisher's exact test or, where appropriate, the standard chi-square test (27).

Multivariate logistic regression was performed with the SPIDA statistical package (28). Occlusion rates were expressed per patients (with one or more distal anastomoses occluded) and per distal anastomosis. The analysis of multiple distal anastomoses in one patient was addressed using a logistic model, with the distal anastomosis (or graft) as the observational unit and adjusting for patient-specific and graft-specific variables according to statistical methods previously reported (18).

Beta-thromboglobulin values in the study patients showed an abnormal distribution, and logarithmic transformation was required to obtain a normal distribution for comparative statistical analysis. However, for the presentation of data in this report, the arithmetic mean values and standard deviations of the raw data have been used to clarify the pattern evident among various groups. All analyses and conclusions arising from this analysis were performed on logarithmically transformed data. The statistical significance of changes in the same variable was evaluated with Student's t test. A p value <0.05 was considered significant.

Results

Patients. A total of 105 patients randomized to receive either aspirin or placebo were studied with postoperative graft angiography and had at least a preoperative beta-thromboglobulin plasma sample prepared; 98 patients completed the 12 month follow-up study and had three or more serial blood samples for beta-thromboglobulin assay. Two
Table 1. Patient Characteristics and Risk Factors

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No.</strong></td>
<td>49</td>
<td>56</td>
</tr>
<tr>
<td><strong>Mean age (range) (yr)</strong></td>
<td>56.7 (39–69)</td>
<td>55.3 (31–69)</td>
</tr>
<tr>
<td><strong>Male (%)</strong></td>
<td>39 (79.6)</td>
<td>47 (83.9)</td>
</tr>
<tr>
<td><strong>Female (%)</strong></td>
<td>10 (19.6)</td>
<td>9 (16.1)</td>
</tr>
<tr>
<td><strong>FH of angina or AMI (%)</strong></td>
<td>31 (63.3)</td>
<td>43 (76.8)</td>
</tr>
<tr>
<td><strong>Hypertension (%)</strong></td>
<td>19 (37.3)</td>
<td>23 (41.1)</td>
</tr>
<tr>
<td><strong>Smoking (%)</strong></td>
<td>34 (69.4)</td>
<td>38 (67.9)</td>
</tr>
<tr>
<td><strong>Angina (NYHA functional class)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I or II (%)</td>
<td>29 (59.2)</td>
<td>37 (66.1)</td>
</tr>
<tr>
<td>III or IV (%)</td>
<td>20 (40.8)</td>
<td>19 (33.9)</td>
</tr>
<tr>
<td><strong>Previous AMI (%)</strong></td>
<td>39 (79.6)</td>
<td>47 (83.9)</td>
</tr>
<tr>
<td><strong>Serum cholesterol (mmol/liter) (± SD)</strong></td>
<td>7.0 ± 1.3</td>
<td>7.3 ± 1.2</td>
</tr>
</tbody>
</table>

AMI = acute myocardial infarction; FH = family history; NYHA = New York Heart Association.

refused to return for follow-up evaluation after leaving the hospital, one of whom had a pulmonary embolism on the 17th postoperative day; one patient moved overseas; one died of carcinoma of the lung; one had a cerebrovascular accident; and two died suddenly within 3 months of operation, the cause of death not being ascertained. Of the seven patients who did not have 12 month angiography, six had A and B plasma samples available and one had an A sample only. These are included in the analysis of early graft and overall graft occlusion.

Of the 105 patients included in the final analysis, 49 received the placebo and 56 received aspirin. Clinical variables and risk factors for coronary artery disease were evenly distributed between the two groups (Table 1). There were 3.6 distal anastomoses per patient in the placebo group and 3.4 per patient in the aspirin group. Endarterectomy was performed in 11% of subjects in the placebo group and 12% of patients in the aspirin group. A total of 363 distal anastomoses were performed overall.

Plasma beta-thromboglobulin. Plasma levels of beta-thromboglobulin were assayed in 105 preoperative (A), 97 1 week postoperative (B), 59 3 month (C) and 86 12 month (D) samples. Throughout the study, control plasma samples were prepared from 51 healthy hospital employees, yielding a mean control value of 22.6 ± 11.1 ng/ml. The preoperative (sample A) beta-thromboglobulin concentration of 29 ± 13.5 ng/ml for the entire group (n = 105) was significantly higher than for the control group (p < 0.004).

Postoperatively, beta-thromboglobulin remained comparatively constant over the subsequent 12 months (Table 2), with the exception of sample B, which was almost invariably elevated further and significantly higher than at other sampling times. The constancy of plasma beta-thromboglobulin levels in individual patients over time is demonstrated in Figure 1. The correlation between plasma beta-thromboglobulin assayed at 3 months (sample C) and 12 months (sample D) in the 43 patients who had both samples available is remarkably close, with the exception of 5 patients (p < 0.001, r = 0.65). In both placebo and treatment groups, the beta-thromboglobulin level tended toward "normal" at 3 and 12 months postoperatively, but these changes were not significant. However, the reduction in plasma beta-thromboglobulin from the preoperative level to that at 12 months postoperatively was greater in the aspirin-treated than in the placebo group when analyzed by the two sample t test (p < 0.001) (Table 2).

Multivariate logistic regression analysis demonstrated a significant association between preoperative (sample A) plasma beta-thromboglobulin concentration and graft occlusion both overall (p < 0.02) and late (p < 0.02). There was no association found with early graft occlusion (p = 0.44).

There was also no significant association between beta-thromboglobulin and serum creatinine, a history of cigarette smoking, gender, hyperlipidemia or preoperative use of calcium antagonists or beta-adrenergic blocking agents. Likewise, a history of previous myocardial infarction from historical, electrocardiographic (ECG) and ventriculographic data did not show any correlation with plasma beta-thromboglobulin concentration. No patients were found to have intracardiac thrombus on the preoperative left ventriculogram. There was no association between the preoperative beta-thromboglobulin level and the maximum of four anticardiolipin antibody levels measured in corresponding patients. There was no correlation between preoperative beta-thromboglobulin levels and D-dimer. Furthermore, no correlation was found between preoperative beta-thromboglobulin level and the number of arteries grafted at surgery.
Table 2. Plasma Beta-Thromboglobulin Levels Preoperatively and During 12 Months of Follow-up

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aspirin Group</th>
<th>Placebo Group</th>
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<tbody>
<tr>
<td></td>
<td>Nonoccluded (n = 49)</td>
<td>Occluded (n = 7)</td>
</tr>
<tr>
<td></td>
<td>29.6 + 14.5</td>
<td>30.3 + 13.1</td>
</tr>
<tr>
<td></td>
<td>39.4 + 16.4</td>
<td>48.9 + 15.4</td>
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<tr>
<td></td>
<td>25.6 + 12.6</td>
<td>27.4 + 10.1</td>
</tr>
<tr>
<td></td>
<td>24.3 + 10.0</td>
<td>28.0 + 10.8</td>
</tr>
</tbody>
</table>

*Aspirin (total samples A-D) versus placebo (total samples A-D) p < 0.001, see text. Values are arithmetic mean values ± SD. Sample A was collected 2 days preoperatively, sample B at 7 days, sample C at 3 months and sample D at 12 months postoperatively.

When stenosis of single vein grafts was assessed on the 1 year angiogram, no association was found between severity of graft narrowing and beta-thromboglobulin level.

**Placebo group.** Of the 49 patients in this group, 17 (34.7%) had one or more graft occlusions (Table 3), 6 early, 10 late and 1 with both an early and a late occlusion. Analysis of preoperative beta-thromboglobulin data in the placebo group (Fig. 2) revealed a significant difference in the preoperative beta-thromboglobulin level between those with graft occlusion (35.8 ± 15.2 ng/ml, n = 17) and those without graft occlusion (24.2 ± 9.0 ng/ml, n = 32) (p < 0.03).

**Aspirin group.** Of the 56 aspirin-treated patients, 7 (12.5%) had one or more graft occlusions, 3 early and 4 late. There was no significant correlation between the preoperative plasma beta-thromboglobulin concentration and graft occlusion in the aspirin-treated group (Fig. 2). The effect of aspirin treatment on the coronary artery bypass graft occlusion rate was significant (p < 0.005). When the numbers of patients with graft occlusion are divided into early and late occlusions and the effects of beta-thromboglobulin level (preoperative sample) and treatment with aspirin are analyzed by logistic regression, the following associations were found: late graft occlusion was significantly associated with the preoperative beta-thromboglobulin level (p < 0.02), and aspirin treatment was effective in preventing occlusion when adjusted for the preoperative beta-thromboglobulin level (p < 0.005).

**Distal anastomoses.** A similar reduction in occlusion rate was achieved with aspirin when total distal anastomoses were analyzed (Table 3).

**Endarterectomy.** The performance of endarterectomy was associated with an independent risk of graft occlusion; 9 of 23 patients had graft occlusion compared with 15 of 82 patients in whom endarterectomy was not performed (p < 0.035). The preoperative beta-thromboglobulin level in patients who underwent endarterectomy was not significantly different from that in those not requiring endarterectomy (29.5 ± 12.1 versus 28.9 ± 13.9 ng/ml, respectively).

**Thrombotic events.** Postoperative thrombotic events (pulmonary embolism, myocardial infarction, cerebrovascular accident, sudden death and peripheral arterial embolism) occurred in similar proportions of the aspirin-treated (16.1%) and placebo (12.2%) groups. The preoperative beta-thromboglobulin level in these 15 patients was not significantly different from that in the remaining study patients.

**D-dimer assay.** Plasma samples for D-dimer assay were collected in 96 patients preoperatively (sample A). Control samples were collected from 45 healthy hospital employees throughout the study. Preoperatively, both treatment groups had significantly higher levels (108 ± 183.9 ng/ml) than those of the control group (64.3 ± 61.85 ng/ml) (p < 0.03). The preoperative D-dimer level in patients who subsequently developed graft occlusion was not significantly different from that of patients who did not.

**Compliance.** On the basis of urine tests, pill counts and tablet record diary entries, 94% of patients took ≥95% of their medications and 2% took <90%. A test for urinary salicylic acid was positive in 90% of the aspirin-treated group and 3% of the placebo group. Platelet aggregation...
studies showed a full "aspirin defect" in all six patients from the treated group and in one of nine from the placebo group.

**Side effects.** Administration of the study medication coincided with hematemesis in one patient (aspirin group) 2 weeks postoperatively, necessitating blood transfusion. There was no significant difference between the aspirin and placebo groups in blood loss over the first 24 h (532 ± 336 versus 475 ± 223 ml) or total blood loss (698 ± 451 versus 592 ± 236 ml). Three of 56 patients receiving aspirin had immediate reoperation for bleeding compared with none of 49 patients taking placebo. In each case, a surgically correctable cause of bleeding was identified.

**Complications.** Occurred in three patients during early postoperative angiography, but there were no significant complications during the 1 year study. Early complications comprised a "shower" of peripheral emboli in the right foot in one patient (treated successfully with intravenous heparinization), traumatic dissection of proximal graft anastomosis in one patient (asymptomatic) receiving aspirin (treated as an occlusion in the data analysis) and severe allergic reaction to meglutaminediatrizoate (resolving promptly with fluid replacement, antihistamine and hydrocortisone intravenously). There were no permanent sequelae from these complications.

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**Figure 2.** The preoperative beta-thromboglobulin (βTG) levels in patients subdivided according to treatment (aspirin [n = 56] or placebo [n = 49]) and the presence or absence of graft occlusion. Mean values ± SD are represented by solid bars. The mean ± SD beta-thromboglobulin levels of the control group are presented on the right of the figure. ● = nonocclusion; ▲ = occlusion.

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**Discussion**

**Plasma beta-thromboglobulin and graft occlusion.** From this study, elevated preoperative plasma beta-thromboglobulin concentration is significantly correlated with occlusion of saphenous vein bypass grafts during the first postoperative year. When the placebo group is analyzed separately, there is an even clearer association between the preoperative beta-thromboglobulin level and the incidence of subsequent graft occlusion (Fig. 2).

**Effect of aspirin on graft occlusion.** Aspirin was highly protective of graft patency, reducing the proportion of patients with graft occlusion from 34.7% to 12.5%. This benefit was more apparent in patients with higher beta-thromboglobulin levels. Earlier reports have shown that aspirin (17,18,20) and other antiplatelet agents (16) improve early graft patency when administration of the drug is commenced either preoperatively or in the early postoperative period.
tive period. Three patients in this study required reoperation and were found to have a surgically correctable cause for bleeding, even though they were in the aspirin-treated group. The beneficial effect of once daily aspirin was also maintained when patency rates at late vein graft angiography were assessed. In a recent report (20) comparing several antiplatelet regimens for coronary artery bypass surgery, a cumulative patency rate for all three aspirin-treated groups was needed to show a significant benefit compared with placebo at late angiography.

**Beta-thromboglobulin levels: effects of surgery and aspirin.** Preoperatively, the mean beta-thromboglobulin level in the study patients with coronary artery disease was significantly higher than that in the control group. Factors associated with increased beta-thromboglobulin (lipidemia and left ventricular thrombosis) showed no significant correlation with beta-thromboglobulin levels in this study. Patients with significant renal impairment, which may contribute to high levels of beta-thromboglobulin, were excluded according to the protocol. The preoperative beta-thromboglobulin level in patients with unstable angina was not significantly higher than that in those with class I or II angina, although there were only a few patients in the former category. Although these results are in accord with several previous studies (2,3,6) showing elevated beta-thromboglobulin in patients with coronary disease, other centers (15) have shown conflicting results. Also, a similar lack of agreement is encountered in studies of platelet survival time in patients with coronary disease. Although there are several reports (8,10) of shortened platelet survival in patients with coronary artery disease, other centers (14) have reported no significant difference from values in the normal population. Reasons for this discrepancy may well reflect differences in techniques for measuring platelet survival in vivo and in the collection, preparation and assay of plasma for beta-thromboglobulin.

The postoperative beta-thromboglobulin (sample B) level was significantly higher than the preoperative and late postoperative levels in all subgroups. Blood was collected immediately before the early angiogram and as late as possible after any invasive vascular procedure. This higher level most likely reflects the combined influences of the cardiopulmonary bypass pump on the circulating platelet pool and the effects of major surgery, as well as the frequent need for intravenous therapy and venipuncture in the first week postoperatively. There was a trend toward a decrease in late postoperative beta-thromboglobulin levels in both groups, the trend being more apparent in the aspirin-treated group, although this did not reach significance. Despite this, the overall decrease in beta-thromboglobulin over 12 months was significantly greater in patients receiving aspirin than in those receiving placebo (p < .001). This trend is in accordance with the findings of an earlier study (3) in which sulfipyrazone rather than aspirin was employed. A significant reduction in plasma beta-thromboglobulin concentration as a result of aspirin treatment in patients with peripheral vascular disease was recently reported (29). The decrease in beta-thromboglobulin was associated with an increase in platelet survival time in both these studies (3,29).

In this prospective study, we observed a significant correlation between elevated plasma beta-thromboglobulin level and a thrombotic event (graft occlusion). Those who developed graft occlusion in the placebo group had the highest mean preoperative beta-thromboglobulin concentration of all subgroups. Although this does not provide unequivocal evidence of a cause and effect relation between beta-thromboglobulin and graft occlusion, the inference could be drawn that patients with biochemical evidence of greater platelet activation have a systemic predisposition to develop thrombosis. Thus, we may be able to identify a subgroup at greater risk of graft occlusion, even though factors such as local anatomy, blood flow and other vasoactive substances will also modify the platelet-vessel wall interaction. Although elevated beta-thromboglobulin is predictive of subsequent graft occlusion, a similar pattern was not observed with respect to other thrombotic events during or after surgery.

**Pathogenesis of graft occlusion.** The pathogenesis of vein graft narrowing after aortocoronary bypass surgery involves a diffuse intimal hyperplasia that has been postulated to be related to the mitogenic effects of platelet-derived growth factor (23). Antiplatelet therapy has been reported (23) to have minimal effect on the severity of this process, and our data support this finding.

**Elevated antithromboglobulins.** Antithromboglobulins have been reported (30) to be associated with a higher rate of thrombotic events in younger men after myocardial infarction. One postulated mechanism to explain this association was that antithromboglobulins cause continuing platelet activation by cross-reacting with antigenically similar phospholipids in the platelet cell wall, predisposing to thrombosis. In an analysis of the first 83 patients entered into this study (24), we reported a strong association between elevated antithromboglobulin antibodies and bypass graft occlusion. However, in this study, we have been unable to show any correlation between antithromboglobulins and beta-thromboglobulin levels. The poor correlation between these two markers of thrombotic tendency and clinical events reflects the multifactorial nature of the interaction of platelets, blood and vessel wall, resulting in thrombosis.

The D-dimer is a relatively new immunoassay to detect the degradation products of cross-linked fibrin (26). Elevated levels have been reported in patients with pathologic fibrinolysis (31) and after intravenous streptokinase therapy for acute myocardial infarction (32). In this study, patients with coronary artery disease were found to have higher D-dimer levels than in the normal population. The preoperative D-dimer level was not significantly different between patients with patent or occluded grafts. These data may
conceivably reflect a delicate equilibrium between ongoing local thrombosis and endogenous thrombolysis within an atherosclerotic coronary system.

Limitations of the study. Plasma samples were collected and prepared for assay by the same physician throughout the study to achieve a high degree of consistency in technique and minimize in vitro release of platelet secretory proteins. It has been suggested (33) that simultaneous assay of both beta-thromboglobulin and platelet factor 4 is necessary to allow distinction between in vivo and artifactual in vitro release. We assayed for beta-thromboglobulin alone in this study and excluded from analysis six disproportionately elevated beta-thromboglobulin levels, each >100 ng/ml. The decision to exclude these elevated levels was based on the presumption that because all six levels were in excess of 4 SD of their respective group mean values (after logarithmic transformation), they reflected aberrant or artifactual in vitro release. Such extreme data would distort rather than clarify the meaningful interpretation of these results. The high degree of reproducibility over time of consistent beta-thromboglobulin levels within 43 individual study patients (Fig. 1) further emphasizes the aberrancy of these very elevated levels.

Conclusions. Our data support the contention that patients with coronary atherosclerotic disease have significantly increased platelet activation. Whether this is a cause or effect of the atherosclerotic plaque remains unclear. However, we have prospectively shown a strong association between a biochemical index of excessive platelet activation and subsequent graft occlusion. Importantly, aspirin (324 mg/day) given early after bypass surgery provides significant protection against vein graft occlusion during the first year after operation. Aspirin is associated with a greater reduction of indexes of platelet activation than is bypass surgery alone and may afford protection against various clinically relevant thrombotic events.

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References


