The increase of plasminogen activator inhibitor activity is associated with graft occlusion in patients undergoing aorto-coronary bypass surgery

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Received 7 March 1997; accepted for publication 1 August 1997

Summary. Early graft occlusion is a common complication in patients undergoing aorto-coronary bypass surgery. Both mechanical and haemostatic factors play a role in the pathogenesis of thrombotic occlusion. Several studies have demonstrated a relationship between fibrinolytic activity and venous or arterial thrombosis. We undertook this study to evaluate the possible contribution of the fibrinolytic system to postoperative occlusion in patients undergoing aorto-coronary bypass graft (CABG).

A venous occlusion (VO) test was performed preoperatively in 82 patients undergoing revascularization procedures. Before and after VO the euglobulin fibrinolytic activity and tissue type plasminogen activator (t-PA) activity and antigen were measured. Plasminogen activator inhibitor (PAI) activity and antigen and fibrinogen were also assessed in the preocclusion sample. An angiography performed 10 d postoperatively showed graft occlusion in 23% of patients. Patients with graft occlusion had significantly higher preoperative PAI activity than patients without occlusion (P < 0.001). Reduced fibrinolytic response and t-PA capacity was also observed in the group of patients with graft occlusion (P < 0.03 and P < 0.02 respectively).

We found a reduced preoperative fibrinolytic response, mainly related to high plasma PAI activity in patients with postoperative graft occlusion. These results suggest that increased PAI activity might have a predictive value for early thrombosis in patients undergoing CABG.

Keywords: aortocoronary bypass, thrombosis, fibrinolysis, PAI-1.

Graft thrombotic occlusion is a common complication in patients undergoing aortocoronary bypass surgery (CABG). Angiographically proven occlusion rates within a month after operation are approximately 20% for all types of vein grafts and about 5% when an internal mammary artery is used. The occlusion rate per patient with one or more distal anastomoses occluded in the same period ranged from 21% to 38% (Hutchings, 1980; Fuster & Chesebro, 1986).

Experimental studies revealed an early phase of thrombotic occlusion, starting in the postoperative period (Chesebro et al., 1986). Known risks factors for early occlusion include low graft blood flow, small luminal size of the grafted vessels, endarterectomy, local atheroma at the arteriotomy site, elevated serum lipids and smoking (Lytle et al., 1985).

Platelets play a pivotal role in the pathogenesis of early graft occlusion. Vein grafts are vulnerable to endothelial damage which may occur by handling during the operation and by sudden exposure to the high-pressure pulsatile arterial system. Platelet deposition occurs in areas of endothelial damage and the consequent release of platelet factors initiates mural or occlusive thrombus formation that begins during the operation (Barboriak et al., 1978; Falk, 1989).

Since fibrin is an important structural component of thrombi, and the fibrinolytic system contributes to the clearance of fibrin from the circulation (Collen & Lijnen, 1991), it can be assumed that the integrity of fibrinolytic mechanisms is a prerequisite for graft permeability. In fact, several authors have convincingly demonstrated a relationship between fibrinolytic activity and either venous or arterial thrombosis (Páramo et al., 1985a; Nilsson et al., 1985; Amsar et al., 1988; Rocha et al., 1988; Engesser et al., 1989). A previous study has also suggested that the impairment of the fibrinolytic system before surgery may play an important role in the pathogenesis of early coronary graft occlusion after revascularization procedures (Arnesen et al., 1983), but it was performed using nonsensitive assays for the different components.
In this study the fibrinolytic potential in patients undergoing CABG was assessed by measuring the preoperative fibrinolytic response to venous occlusion as well as the plasma concentration of fibrinolysis activators and inhibitors, using highly sensitive assays, and results were correlated with postoperative graft occlusion.

PATIENTS AND METHODS

Patients. The study was carried out in 82 patients consecutively admitted for elective aorto-coronary bypass operations because of severe stable angina pectoris. There were 76 males and six females with a mean age of 59 ± 9 years. The exclusion criteria were: valvular heart disease, emergency surgery and patients undergoing a different surgical procedure simultaneously such as ventricular aneurysmectomy or valvuloplasty. None of the patients received anticoagulant or corticosteroid medication. Oral antiplatelet agents had been discontinued a week before the operation. All patients received standard antithrombotic prophylaxis with dipyridamole throughout the postoperative period. Patients received anticoagulant or corticosteroid medication. Oral antiplatelet agents had been discontinued a week before the operation. The saphenous vein was used in 136 (63%) and the mammary artery in 80 (37%) patients. Smoking, hypercholesterolaemia, hypertension, and diabetes mellitus were considered: smoking, hypercholesterolaemia, hypertension, and diabetes mellitus.

A group of 30 age-matched healthy subjects served as a reference population for control values.

Blood sampling. Blood samples were drawn by venepuncture between 8.00 and 9.30 a.m. after the subjects had rested in the supine position for 10 min and after venous stasis produced by a tourniquet applied to the upper arm inflated to a pressure of 100 mmHg (between systolic and diastolic pressures) for 10 min. Samples were taken at least 2 h before surgery. No acute episode of coronary insufficiency was present at the time of blood collection. Pre- and postocclusion blood was collected into 0.13 M trisodium citrate in a ratio 9:1, centrifuged at 2000 g for 20 min at 4°C and the platelet-poor plasma was stored at −70°C. To measure t-PA activity, 1 ml of citrated whole blood was acidified by mixing with 1 ml of acetate buffer (0.2 M, pH 3.9). This was immediately centrifuged at 2000 g for 15 min, and 0.6 ml of supernatant were further acidified by the addition of 40 μl of 1 M HCl and then frozen at −70°C.

Methods. Before and after VO the following parameters were measured: (i) Euglobulin fibrinolytic activity (EFA) on fibrin plates. Euglobulin fractions for fibrin plates were prepared by mixing 0.5 ml of plasma with 4.5 ml 0.025% chilled acetic acid. Incubated on ice for 15 min and then centrifuged at 3000 g for 10 min at 4°C. The precipitate was then resuspended in 0.5 ml phosphate-buffered saline. This fraction was applied to fibrin plates in triplicate and the diameter of the area of lysis measured after 18 h at 37°C (Kluft et al., 1976). Results were expressed as U/ml by reference to a standard curve produced using the International standard for t-PA (ref. 86/679, kindly provided by Dr Gaffney, NIBSC, London). The fibrinolytic response was defined as the difference between postocclusion and preocclusion values of EFA as described by Stalder et al. (1985). (ii) t-PA activity was measured in acidified plasma samples by an amidolytic microtitre assay (Coatest t-PA, KabiVitrum, Stockholm, Sweden). t-PA activity release was defined as the difference between activity before venous occlusion and the activity after venous occlusion. (iii) The level of t-PA antigen was determined by an ELISA assay using a monoclonal antibody against t-PA with a commercially available kit (tintElize t-PA from Biopool, Sweden) (Korninger et al., 1986). t-PA antigen release was calculated as the difference between postocclusion and preocclusion values of t-PA Ag (Hamsten et al., 1985).

Before VO the following parameters were also determined: (i) PAI activity was measured by adding a certain amount of t-PA to diluted plasma and determining residual t-PA activity as described by Chmielewska et al. (1983). Inhibitor activity was expressed in units of PAI-1 antigen per ml. (ii) PAI-1 antigen was determined by an ELISA assay essentially as described by Declerk et al. (1988) using a monoclonal antibody against PAI-1 (tintElize PAI-1 from Biopool, Sweden). (iii) Fibrinogen was measured by the Clauss (1957) method. (iv) Whole serum cholesterol, HDL and LDL cholesterol and triglycerides were measured by standard enzymatic methods.

Angiographic analysis. Selective shunt angiography using the Judkins technique was performed in all patients on postoperative day 10 to evaluate the permeability of coronary grafts. The angiographic results were analysed by an independent team who were unaware of the fibrinolysis findings. The results were classified as total occlusion in at least one graft or no occlusion.

Statistical analysis. Results are expressed as mean ± SD. Since the values were not distributed normally, the Mann-Whitney test after log transformation was used for group comparison. For group differences regarding the fibrinolytic potential, the exact Fisher test was used. A P value < 0.05 was considered to be significant.

RESULTS

Eighty-two patients undergoing CABG were included in this study. Angiographically proven graft occlusion was demonstrated in 19 patients (23%). Table I shows the total number and the type of graft. The global rate of graft occlusion was

| Table I. Graft occlusion in relation to type of graft. |
|-----------------|-----------------|
|                  | No. of grafts   | Occluded |
| Saphenous vein   | 136             | 22 (16.1%) |
| Mammary artery   | 80              | 3 (3.7%)  |
| Total grafts     | 216             | 25 (11.5%) |

11.5% and it was significantly higher for saphenous grafts (16.1%) as compared to mammary artery grafts (3.7%) (P<0.0001).

**General assessment and risk factors evaluation**

As shown in Table II, no significant differences with regard to age, diabetes, hypercholesterolaemia, including both HDL and LDL cholesterol, hypertriglyceridaemia, hypertension and smoking were observed in patients with graft occlusion when compared to those without graft occlusion. Although the rate of graft occlusion was higher in women than in men (50% vs 21%), this difference was not significant due to the small number of women included.

**Baseline fibrinolytic activity in patients with and without graft occlusion**

Preoperatively, no differences for the fibrinolysis parameters analysed were observed in the group of patients compared to healthy subjects (not shown), except for the concentrations of PAI, both functional and antigen, which were significantly higher (P<0.001) in the group of patients (12.5 ± 8.8 U/ml and 30.7 ± 19.6 ng/ml) than in controls (6.5 ± 4.8 U/ml and 12.9 ± 9.6 ng/ml).

Table III shows the preoperative fibrinolysis parameters before and after VO in relation to the postoperative angiographic findings. The mean plasma PAI-1 activity was significantly higher in patients with postoperative graft occlusion (20.9 ± 10.1 U/ml, 95% CI 16.0–25.8) than in those without graft occlusion (10.8 ± 7.6, 95% CI 8.9–12.7) (P<0.001). Fig 1 shows the distribution of PAI-1 activity in both patient groups. 13/19 patients with graft occlusion (68%), but only 13/63 patients (20%) without graft occlusion, had plasma levels of PAI-1 higher than the mean ± 2 SD of normal values.

No differences in the distribution of other haemostatic fibrinolysis parameters analysed before venous occlusion were observed between groups.

**Postocclusion fibrinolysis parameters in patients with and without graft occlusion**

After venous occlusion, EFA was significantly lower in patients with graft occlusion (1.7 ± 1.5 U/ml v 3.8 ± 5.0 U/ml, P<0.05). t-PA activity was significantly reduced in this group, compared to patients without graft occlusion (4.7 ± 3.4 U/ml v 9.1 ± 7.6 U/ml, P<0.001), whereas t-PA antigen concentration was similar between the groups (18.4 ± 14.8 ng/ml v 19.3 ± 14.0 ng/ml).

In order to establish which of the fibrinolysis parameters was best associated with early graft occlusion we calculated the fibrinolytic response, t-PA activity release and t-PA antigen release in both patient groups. As shown in Table IV, the fibrinolytic response and t-PA activity release were significantly reduced in patients with postoperative graft occlusion (P<0.03 and P<0.02 respectively), whereas the

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Table II. Baseline characteristics, risk factors and biochemical profile.

<table>
<thead>
<tr>
<th></th>
<th>Patients without occlusion (n=63)</th>
<th>Patients with occlusion (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.0 ± 9.0</td>
<td>59.2 ± 9.6</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>60/3</td>
<td>16/3</td>
</tr>
<tr>
<td>Diabetes</td>
<td>9 (14%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>28 (44%)</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>Smoking</td>
<td>39 (62%)</td>
<td>10 (53%)</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.72 ± 0.98</td>
<td>5.37 ± 1.06</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.63 ± 0.61</td>
<td>1.65 ± 0.53</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>0.90 ± 0.33</td>
<td>0.84 ± 0.20</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>4.22 ± 0.92</td>
<td>3.91 ± 0.88</td>
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</tbody>
</table>

Table III. Fibrinolytic parameters before venous occlusion (VO) in patients with and without graft occlusion. Mean ± SD is reported.

<table>
<thead>
<tr>
<th></th>
<th>Patients without occlusion (n=63)</th>
<th>Patients with occlusion (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFA (U/ml)</td>
<td>0.8 ± 0.9</td>
<td>1.0 ± 1.2</td>
</tr>
<tr>
<td>t-PA activity (U/ml)</td>
<td>2.1 ± 1.2</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>t-PA antigen (ng/ml)</td>
<td>7.1 ± 3.7</td>
<td>7.9 ± 5.9</td>
</tr>
<tr>
<td>PAI activity (U/ml)</td>
<td>10.8 ± 7.6</td>
<td>20.9 ± 10.1</td>
</tr>
<tr>
<td>PAI-1 antigen (ng/ml)</td>
<td>30.1 ± 18.0</td>
<td>34.1 ± 26.2</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.5 ± 0.7</td>
<td>3.8 ± 0.9</td>
</tr>
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</table>

NS: not significant.
t-PA antigen release showed no differences between groups. When analysing the PAI-1 levels in patients with either lower fibrinolytic response and/or t-PA activity release we found significantly higher (mean ± 2 SD) levels in 29/33 of these patients (87.8%) which indicated that increased PAI activity was the main cause of the low fibrinolytic response in this group. In the 33 patients with low fibrinolytic potential the graft occlusion rate was 45% (15/33), compared to 8% (4/49) in the remaining patients.

**DISCUSSION**

This study showed that a low preoperative fibrinolytic potential, mainly due to high PAI-1 levels, significantly contributed to early graft occlusion in patients undergoing CABG. In our series the rate of graft occlusion (23%) was higher for saphenous grafts than for mammary artery grafts, which is in agreement with previous reports (Chesbro et al. 1986). This difference could be explained by the anatomical and physiological characteristics of grafts.

Different studies have indicated that an impairment of both coagulation and fibrinolysis are implicated in coronary heart disease (Hamsten, 1993). It remains to be ascertained whether disturbances of haemostatic function predispose to early graft occlusion. In a previous report we demonstrated that the preoperative increase of thrombin–antithrombin complexes was higher in patients with graft occlusion than in those with graft patency, suggesting that a greater generation of thrombin before surgery can be an important factor in the development of thrombosis after CABG (Rifon et al., 1994). Studies on fibrinolytic function in connection with surgery have been conducted mainly in patients undergoing abdominal or orthopaedic surgery, emphasizing the existence of a postoperative fibrinolytic shutdown secondary to increased PAI activity (Páramo et al., 1985a; D’Angelo et al., 1985; Kluff et al. 1986; Rocha et al., 1988). Few clinical studies have addressed the preoperative fibrinolytic activity in patients subjected to CABG. Arnessen et al. (1983) showed low preoperative fibrinolytic activity to be associated with graft occlusion. More recently, Moor et al. (1994) examined the time course of individual fibrinolytic components in a small series of patients showing weak associations with the presence of graft occlusion.

We have determined the fibrinolytic response to venous occlusion in a large series of patients undergoing CABG. When compared to a control group we found significantly higher PAI activity in the study population, confirming previous reports of an association between coronary artery disease and high PAI levels (Páramo et al. 1985b; Thompson et al., 1993; Rocha & Páramo, 1994).

Basal PAI activity was significantly higher in patients with graft occlusion compared to those with graft patency, whereas no differences in other fibrinolysis parameters analysed were observed. After VO a lower increase of EFA and t-PA activity was observed in patients with graft occlusion. Consequently the fibrinolytic response and t-PA activity release were significantly lower in patients with graft occlusion. The plasma PAI-1 activity in patients with low fibrinolytic potential (either low fibrinolytic response and/or low t-PA activity release) was significantly higher than in those with normal fibrinolytic potential, thus indicating that PAI-1 is main cause of fibrinolysis impairment. The fact that only PAI activity differed whereas both PAI-1 and t-PA antigen were similar in patients with and without graft occlusion could be due to the different origin of PAI-1 (endothelial cells, platelets, etc.) although an interference of other inhibitors cannot be ruled out.

Alterations in the balance between the coagulation and the fibrinolysis mechanisms can play an important role for the development of thrombosis within the arterial vessels. Of importance is the pathophysiological role of hypercoagulability (increase of fibrinogen and factor VII) and deficient fibrinolysis (mainly related to high PAI-1) in patients with coronary disease (Hamsten, 1993; Puster et al. 1992). Mean fibrinogen levels were slightly higher in patients with graft occlusion, but this difference was not significant in our study. Likewise, the fact that lipid concentrations were similar between groups suggest that they do not contribute significantly to early graft occlusion. It must be emphasized, however, that the lipoprotein(a) level, not measured in our samples, has been found to be a predictor of stenosis after CABG (Hoff et al. 1990).

Several considerations can be made regarding the association between the accelerated thrombotic disease and PAI activity elevation. The association may be a reflection of an underlying process responsible for increased PAI, such as the insulin resistance syndrome (Juhan-Vague et al. 1989, 1993; Schneider et al. 1993). In our population the insulin levels were not determined and so the presence or absence of insulin resistance was not assessed, but the proportion of diabetic patients was similar in the two groups. Likewise, no differences in the triglyceride levels were observed between patients with or without graft occlusion. Alternatively, the possibility that PAI elevation affects the progression of vascular disease in patients predisposed to accelerated atherosclerosis and restenosis should also be considered (Fujii et al. 1992). Finally, the association may be causal but attributable to changes in the vessel wall involving expression of fibrinolytic proteins associated with elaboration of excessive amounts of PAI-1. The possibility of measuring PAI-1 expression in the saphenous veins might also be considered in the light of the role of local expression in atherosclerosis (Schneiderman et al. 1992).
In conclusion, we have shown that low preoperative fibrinolytic activity, related to high PAI activity, may play a significant role for early graft occlusion in patients undergoing CABG. Whether PAI activity is also a marker for late graft occlusion needs to be evaluated. The possibility of reducing PAI activity levels may represent another approach to be taken into consideration to prevent graft thrombosis related to revascularization procedures.

ACKNOWLEDGMENT
This work was supported by a grant 89/0448 from the FIS of the Ministerio de Sanidad. Spain.

REFERENCES.
thrombosis despite prophylaxis in total hip replacement. Thrombosis and Haemostasis, 59, 93–95.