Plasma levels of coagulation inhibitors, fibrinolytic markers and platelet-derived growth factor-AB in patients with failed hip prosthesis

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ABSTRACT We studied the plasma levels of coagulation inhibitors, of fibrinolysis and PDGF-AB, in patients with aseptic loosening of the hip replacement. 23 patients having loose hip prostheses were compared to patients having 15 stable hip prostheses, and 26 undergoing primary hip replacement. The levels of the coagulation inhibitors antithrombin III and protein C were determined by chromogenic assays. Fibrinolysis was evaluated by the changes in fibrin degradation products (D-dimer), determined by enzyme immunoassay, and in the plasminogen activator inhibitor-1 (PAI-1), by enzymatic assay. PDGF-AB was determined by enzyme immunoassay. In patients with failed prostheses, we found fibrinolysis activation, as shown by a statistically significant increase in D-dimer and a significant decrease of PAI-1. No significant differences were obseved in antithrombin III, protein C, and PDGF-AB. PAI-1 and D-dimer assays in failed prostheses may be useful for the pathogenetic evaluation, because the continuous inflammatory stimulus associated with fibrin deposition may also affect the systemic levels.

In aseptic THR loosening, local inflammation may increase activation of the coagulative pathway by tissue factor release from macrophages. Activation of hemostasis in inflammation causes the deposition of fibrin. D-dimer is a marker of fibrin generation. Coagulation inhibitors, such as antithrombin III and protein C, have antinflammatory properties too (Minnema et al. 2000, Esmon 2001). Plasminogen activator is inhibited by plasminogen activator inhibitor type-1 (PAI-1), which affects not only fibrinolysis, but also wound healing and vascular remodeling. The plasma concentration increases in conditions associated with high levels of interleukin-1 or other cytokines (Tietze et al. 1998). Moreover, higher levels of PAI-1 may stimulate fibrosis, thereby probably inhibiting protease or stimulating the migration of collagen-producing cells into the damaged tissues (Loskutoff and Quigley 2000).

Many of these mechanisms are also involved in the inflammation associated loosening of aseptic prostheses. It has been thought that fibrinolysis may play a role in extracellular matrix degradation in periprosthetic tissues. Macrophages containing phagocytosed material express plasminogen activators, which suggests that undegradable microdebris can initiate and perpetuate a proteolytic activation cascade (Nordsletten et al. 1996). Plasminogen activators, together with other serine proteinases and metalloproteinases, have been found in the interface and pseudocapsular tissues (Takagi 1996).

Growth factors, released by inflammatory cells, can increase graft incorporation (Nimni 1997), but in excessive amounts, they can cause hyperplasia of undesirable cell types. It has been suggested that platelet-derived growth factor (PDGF) is involved in a periprosthetic reaction (Jiranek et al. 1993) and in the pathogenesis of prosthetic

Table 1. Some data concerning patients with loose prostheses (group A), stable prostheses (group B), and before surgery (group C)

Group	A	B	C
Sex			
Women	15	9	18
Men	8	6	8
Age, mean	69	58	59
range	41–81	31–75	30–85
Diagnosis			
Primary osteoarthrosis	20	11	20
Congenital hip dysplasia	2	3	4
Trauma	1	1	–
Osteonecrosis	–	–	2
Follow-up (years), mean	8	4	–
range	0.5–21	4–5	

Table 2. Types of hip prostheses in groups A (23 patients with loose prostheses) and B (15 stable prostheses)

	Group A	Group B
Cement		
Cemented	16	2
Cementless	7	13
Bearing		
Metal-on-metal	3	10
Metal-on-polyethylene	9	1
Metal-on-ceramic	2	–
Ceramic-on-ceramic	4	4
Ceramic-on-polyethylene	2	
Endoprosthesis	3	–

loosening (Goodman et al. 1996). PDGF-A and PDGF-B chain-containing cells have been found in synovial-like membranes from the implant or cement-to-bone interface, and the pseudocapsule from patients surgically treated for aseptic loosening, especially in macrophages with phagocytosed particulate debris, but to some extent also in fibroblasts and in endothelial cells (Xu et al. 1998).

We studied whether a subclinical, but systemic activation of clotting and fibrinolysis, which is easily shown in peripheral blood, was associated with loosening of the hip prosthesis. In particular, we determined the plasma levels of the coagulation inhibitors, antithrombin III and protein C, fibrinolysis inhibitor PAI-1 and PDGF-AB, in patients with loosening of the hip replacement.

Patients and methods

Patient selection

We evaluated 3 groups of patients admitted to the Rizzoli Orthopedic Institute in Bologna. The first group (group A) included 23 patients with aseptic loosening of the hip replacement. Group B included 15 patients with stable prostheses, but without clinical and radiographic signs of loosening. The Harris score ranged from 90 to 98. Group C included 26 patients undergoing primary hip replacement (Table 1). The mean age of group A was higher than the mean ages of group B ($p = 0.02$) and group C ($p = 0.02$), but the laboratory assays were chosen so as not to be affected by these variables. The mean age of group B was similar to that of group C. In group A, 16/23 prostheses were cemented; in group B, only 2/15 prostheses were cemented (Table 2). 7 patients in group A, 4 in group B and 5 in group C were taking nonsteroidal antinflammatory drugs ($p = 0.6$).

A personal history was obtained from each participant, including details concerning previous orthopedic surgery and drugs taken. In the three groups of patients, the hip prosthesis was implanted more frequently because of primary osteoarthrosis and less frequently because of congenital hip dysplasia, trauma, and osteonecrosis of the femoral head. Patients with rheumatoid arthritis were excluded from the study, as also were patients with a suspected periprosthetic infection on the basis of clinical, laboratory (increases in ESR and reactive C protein) and scintigraphic data (scintigraphy with ^{111}indium-labeled granulocytes). Patients with severe heart disease, or on estrogen or oral anticoagulant therapy were also excluded from the study, because these conditions might have affected the results of the tests. Those with resistance to activated protein C were excluded. This was determined by APTT in the presence of an excess of factor V-deficient plasma and activated protein C (Coatest APC Resistance V-S, Chromogenix, Instrumentation Laboratory, Milan, Italy).

Sample collection

Fasting blood was collected in test tubes, containing 0.129 M trisodium citrate anticoagulant (9

Table 3. Coagulation inhibitors, fibrinolysis markers, and plasma PDGF-AB in the patients with loose prostheses (group A), stable prostheses (group B), and before surgery (group C). Values are mean (SEM)

Test, unit of measure	Group A (n 23)	Group B (n 15)	P-value (A vs. B)	Group C (n 26)	P-value (A vs. C)
Antithrombin III, %	99 (2.2)	101 (2.0)	0.3	100 (2.5)	0.9
Protein C, %	108 (4.0)	114 (6.6)	0.6	112 (3.4)	0.3
D-dimer, ng/mL	132 (21)	42 (8.5)	0.01	57 (13)	0.005
PAI-1, U/mL	2.3 (1.1)	8.1 (1.8)	0.02	4.1 (0.94)	0.2
PDGF-AB, ng/mL	2.4 (0.35)	1.9 (0.23)	0.4	2.8 (0.43)	0.6

parts of patient's blood to 1 part of citrate) in an ice-water bath. The samples were centrifuged for 10 minutes at 3000 r.p.m., then the plasma was collected and centrifuged at 11000 r.p.m. for 10 minutes. Both centrifugations were done at +4 °C. The plasma was aliquoted and stored at –80 °C.

Coagulation inhibitors

Antithrombin III functional activity was determined by chromogenic assay (Coamatic Antithrombin Chromogenix, Instrumentation Laboratory, Lexington, MA, USA). Protein C functional activity was detected by chromogenic assay after activation with the venom of Agkistrodon contortrix (Accucolor Protein C, Sigma, St. Louis, MO, USA).

Fibrinolysis

D-dimer was measured by enzyme immunoassay (Dimer test Gold EIA Kit, AGEN Biomedical Ltd., Brisbane, Australia). The inhibitory capacity of plasminogen activator inhibitor-1 (PAI-1) was measured by adding exogenous plasminogen activator (t-PA) to the samples and quantifying the effect of PAI-1 by measuring the reduction in the function of tPA with an enzymatic assay (PAI-1 Activity Kit, Technoclone, Vienna, Austria).

PDGF-AB

PDGF-AB was determined by enzyme immunoassay. The wells were coated with a mouse monoclonal antibody specific for PDGF-AA, binding PGDF of the standards and samples. A polyclonal antibody linked to peroxidase, specific for PDGF-BB, was used as the conjugate. The minimum detectable dose was 8.4 pg/mL (Quantikine Human PDGF-AB Immunoassay, R&D Systems, Minneapolis, MN, USA).

Statistics

All the samples were analyzed in duplicate. Arithmetic mean and standard error were calculated for each continuous variable. The comparison among the groups was made by the Kruskal-Wallis test; the comparison between the couples was made by the Mann-Whitney U-test. Correlations between laboratory tests and age were evaluated by Spearman's test. Differences between nominal variables (nonsteroidal anti-inflammatory drug use) were assessed by the chi-square test. The level for significance was taken as $p < 0.05$.

Results

Coagulation inhibitors

The levels of antithrombin III and protein C activity were similar in the three groups of subjects (Table 3). We found no correlations between antithrombin III or protein C with age (antithrombin III: $\rho = –0.2$; $p = 0.06$; protein C: $\rho = 0.1$; $p = 0.3$). Gender had no effect on antithrombin III ($p = 0.2$) or protein C ($p = 0.7$). The use of nonsteroidal anti-inflammatory drugs did not affect antithrombin III ($p = 0.4$) or protein C ($p = 0.3$).

Fibrinolysis

Some differences were noted in the D-dimer levels among the 3 groups of subjects ($p = 0.009$) (Table 3). The Mann-Whitney U-test showed that group A had significantly higher levels than those in groups B and C, but similar levels in groups B and C ($p = 0.9$). We found no correlation with age ($\rho = 0.2$; $p = 0.4$). D-dimer was not affected by gender ($p = 0.5$), or use of nonsteroidal anti-inflammatory drugs ($p = 0.2$). In the group with implant failure

(group A), plasma levels of PAI-1 were lower than in the group with stable implants (group B) ($p = 0.02$). Moreover, in the former group, PAI-1 levels were lower than in the presurgery patients (group C), but in those with stable implants, they were higher. However, the differences between groups A and C ($p = 0.2$) and between groups B and C ($p = 0.09$) were not significant. PAI-1 showed no correlation with age ($\rho = –0.2$; $p = 0.2$), was not affected by gender ($p = 0.5$), or use of nonsteroidal anti-inflammatory drugs ($p = 0.8$).

PDGF-AB

The levels of PDGF-AB in plasma were about the same in the 3 groups of subjects (Table 3). PDGF-AB showed no correlation with age ($\rho = 0.1$; $p = 0.3$), and was not affected by gender ($p = 0.9$), or use of nonsteroidal anti-inflammatory drugs ($p = 0.8$).

Discussion

Wear of prosthetic materials is associated with an inflammatory reaction. Macrophages activated by the phagocytosed wear particles can produce proteolytic and fibrinolytic enzymes, such as tissue plasminogen activator (Nordsletten et al. 1996). We therefore studied the clotting and the fibrinolytic pathways in patients having loose prostheses to determine whether systemic changes occurred, even at a subclinical level.

The levels of antithrombin III and protein C in the patients with loose prostheses were similar in patients with stable implants and in those about to undergo surgery. Therefore, this phase of coagulation in plasma is not activated in patients with loose prostheses.

However, fibrinolysis was activated in patients with loosening of prostheses, as shown by the higher levels of D-dimer and lower levels of inhibitor PAI-1, than in the other groups. D-dimer generation and fibrinolysis activation can not have been caused by thrombotic events, which occurred independently of loosening because we excluded patients with severe heart disease, on estrogen or oral anticoagulant therapy, or with resistance to activated protein C. Moreover, the patients had normal levels of antithrombin III and protein C, which ruled out a thrombotic event. Although D-dimer is a marker of thrombin generation, thrombin formation in patients with loose prostheses is probably confined to the periprosthetic tissues and does not reduce the levels of antithrombin III and protein C in plasma. Moreover, the determination of D-dimer is more sensitive than that of coagulation inhibitors.

The higher levels of D-dimer in the patients with loose prostheses have been caused by the continuous stimulus of the proteolytic activation cascade in macrophages initiated by undegradable microdebris. Some authors have shown increased activity of serine proteases, such as elastase, around loose hip prostheses, which may weaken periprosthetic tissues (Takagi et al. 1995). Interleukin-6 and interleukin-1, released by macrophages and other cells in periprosthetic tissues, may activate thrombin, and osteoclasts, and thereby contribute to fibrin formation. The proteolytic enzymes released by inflammatory cells may activate plasmin. It has been shown that plasminogen activators are involved in bone resorption, via the degradation of noncollagenous proteins inthe organic matrix of bone (Daci et al. 1999).

In patients with prosthesis loosening, we found lower levels of the fibrinolysis inhibitor PAI-1, and fibrinolysis activation. The subjects with failed implants had PAI-1 levels lower not only than in those with stable prostheses, but also than in those who were about to undergo implant surgery. Therefore, in our opinion, patients with loosened prostheses had persistent fibrinolysis as shown by the reduction in PAI and increase in D-dimer. In patients with stable prostheses, however, fibrinolysis inhibitor levels, such as PAI, are higher and can oppose the activation of proteolytic enzymes. The increase in PAI-1, and therefore the reduction in fibrinolysis, was associated with D-dimer levels similar to those in the preimplant subjects.

Since the PDGF-AB plasma levels in the patients with prosthesis loosening resembled those in the other groups, loosening does not seem to be associated with a greater release of PDGF-AB. In other studies, PDGF mRNA was found in macrophages and fibroblasts of membranous tissue obtained from the cement-bone interface of polyethylene acetabular components that have been revised for aseptic loosening (Jiranek et al. 1993). Thus,

it has been thought that PDGF caused hyperplasia of the connective tissue around the prosthesis. In the present study, we did not determine PDGF-AB in the retrieved tissues. Even if this growth factor is released locally, it probably does not enter the blood stream and the level in plasma should not increase.

Hardly any reports are available about clotting activation in failed hip prostheses. Local and systemic coagulation and fibrinolysis abnormalities have been studied more in inflammatory diseases, such as rheumatoid arthritis. An increase in PAI-1 production and decrease in D-dimer with the increase in inflammatory activity, and a positive correlation between PAI-1 and TNF-α or IL-6 were shown (Kamper et al. 2000). Moreover, a correlation was found between the PAI-1 antigen levels and the endothelial adhesion molecules, especially soluble E-selectin and ICAM-1 (Wållberg-Jonsson et al. 2002). Since PAI-1 is produced by endothelial cells, it was thought that endothelial activation occurred in rheumatoid arthritis, and contributed to the thrombotic process. Peri- and intrasynovial accumulation of coagulation cascade constituents was believed to contribute to the chronicity of the local inflammatory process seen in rheumatoid arthritis. It was postulated that the in situ presence of fibrin by-products may increase thickening of the synovium and formation of the joint effusio. The plasminogen-plasmin enzymatic pathway was implicated as having proteolytic activity in the destruction of joint tissues, directly via its plasmin components or indirectly by activating metalloproteinases. Moreover, a systemic subclinical clotting activation was demonstrated in rheumatoid arthritis, with high levels of D-dimer, thrombin-antithrombin complexes, and plasmin-α_2-antiplasmin complexes and a significant correlation with a marker of collagen metabolism, the type III procollagen peptide (Gabazza et al. 1994).

In failed hip prostheses, subclinical activation of clotting, and especially of fibrinolysis, was shown by the increase in the levels of D-dimer and the decrease in PAI-1 activity. Therefore, while in rheumatoid arthritis, the increase in inflammatory activity is associated with a decrease in fibrinolysis, in failed hip prostheses the opposite condition seems to occur—i.e., hyperfibrinolysis.

However, PAI-1 and D-dimer assays in failed prostheses may be useful for a pathogenetic evaluation of the contribution of fibrinolysis to the loosening. However, they are not really useful as a diagnostic tool because they change in conditions associated with hypercoagulability.

We conclude that coagulation inhibitors and PDGF-AB do not change in patients with aseptic loosening of the hip prosthesis. On the contrary, fibrinolysis is activated, as shown by the increase in D-dimer and the decrease in the inhibitor PAI-1. This increase, which is not caused by previous activation of the plasma phase of coagulation, may be induced by a continuous inflammatory stimulus in the periprosthetic tissue, associated with deposition of fibrin.

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