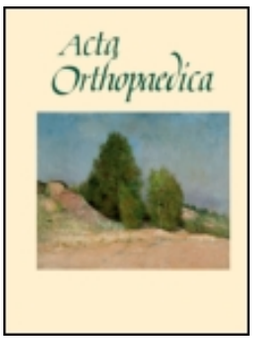

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Plasma levels of platelet-derived growth factor BB and transforming growth factor in patients with failed hip prostheses

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Background **The role of growth factors in prosthesis loosening is unclear. We evaluated the levels of platelet-derived growth factor BB (PDGF-BB), transforming growth factors β1 (TGF-β1) and β2 (TGF-β2), both before and after activation, in patients with aseptic loosening of their hip prosthesis.**

Patients and methods **26 patients with loosened hip implants were compared with 21 patients who had stable hip prostheses, and 28 patients undergoing primary hip replacement. The plasma levels of the growth factors were analyzed by enzyme immunoassay. TGF-β1 and TGF-β2 were determined both before and after activation.**

Results **Patients with aseptic loosening had significantly lower PDGF-BB levels than patients undergoing primary hip replacement, and significantly lower TGF-β2 levels than patients with a stable implant. Patients with stable prostheses had significantly higher TGF-β1 and TGF-β2 levels than patients undergoing primary hip replacement.**

Interpretation **It is possible that the prosthetic implant itself causes a local increase in PDGF-BB, TGF-β1 and TGF-β2, released by osteoblasts and other cells in the microenvironment. The plasma PDGF-BB measured does not correspond to local release, which is probably due to local consumption or degradation. The consumption of PDGF-BB is low in stable implants, and TGF-β1 and TGF-β2 levels increase during bone formation. In loosening, PDGF-BB consumption is higher and causes a significant reduction in plasma levels as compared to presurgery. The formation of poor-quality bone may be related to the scarce increase in TGF-β1 and TGF-β2. In conclusion, compared with patients with a stable implant, a reduction in bone-forming growth factors appears to occur in individuals with aseptic loosening.**

Aseptic loosening is the most common cause of late failure of hip replacement. Wear particles induce a chronic inflammatory response, with release of osteoclast-activating cytokines, which can contribute to weakening of the surrounding bone (Haynes et al. 1998, Jones et al. 1999). Cytokines may also determine an imbalance in the release of bone-forming factors from the cells in the periprosthetic tissues.

Platelet-derived growth factor (PDGF) is present in platelet α-granules, in endothelial cells, and in normal and malignant osteoblasts (Canalis and Rydziel 1996). PDGF-BB is generally considered to be more effective than PDGF-AA in stimulating biological activity, and it has been shown that PDGF-BB has a mitogenic activity on the skeleton (Mitlak et al. 1996). PDGF is thought to have a role in the metabolism of the synovial lining: together with TGF-β, it modulates fibronectin fibrillar matrix formation by synovial fibroblasts (Sarkissian and Lafyatis 1998). It has been proposed that PDGF is involved in periprosthetic response (Jiranek et al. 1993) and in the pathogenesis of prosthetic loosening (Goodman et al. 1996). PDGF-A and PDGF-B

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chain-containing cells have been found in synovial-like membranes from the implant or cement-to-bone interface and pseudocapsule from patients surgically treated for aseptic loosening—particularly in macrophages with phagocytosed debris, but to some extent also in fibroblasts and endothelial cells (Xu et al. 1998). It has been supposed that in aseptic loosening, there is insufficient degradation of PDGF-receptor complex, thus contributing to the accumulation of connective tissue (Niissalo et al. 2002).

Transforming growth factor-β1 (TGF-β1) is an anti-inflammatory cytokine which modulates tissue repair. The most important sources of TGF-β1 are bone and platelet α-granules, but it is produced by many cell types including fibroblasts and macrophages. TGF-β1 and the isoform TGF-β2 produced by osteoblasts are stored in the extracellular matrix as a latent form linked to TGF-β-binding protein-1. TGF-β1 stimulates early differentiation of osteoblast-like cells, including alkaline phosphatase activity, but blocks terminal differentation (Bonewald 1996). TGF-β1 also favors the osteoclastogenic response of hematopoietic precursors (Quinn et al. 2001), and is a potent chemoattractant for osteoclasts (Pilkington et al. 2001).

We have investigated the levels of PDGF-BB and TGF isoforms β-1 and β-2 in the plasma of patients with aseptic loosening of hip prostheses.

Patients and methods

Patient selection

We evaluated 3 groups of patients. The first group included 26 patients (16 women) with aseptic loosening of their hip prosthesis. The mean age was 68 (39–81) years, and the mean follow-up time from the implant was 8 (0.5–21) years. These patients were compared with 21 individuals (12 females) with stable prostheses, without clinical and radiological signs of loosening. The mean age of the patients with stable prostheses was 57 (31–75) years. The Harris score ranged from 90 to 98. The mean follow-up from implantation was 4.5 (4–5) years. The patients with loosened implants and those with stable prostheses were also compared with 28 patients (20 women) undergoing primary hip replacement. The mean age of the latter group was 59 (30–85) years. In the three groups of patients, the most common reason for implanting hip prostheses was primary osteoarthrosis (17 cases in the loosened prosthesis group, 17 cases in the group with stable prosthesis, and 21 cases in the pre-surgery group). Other less frequent indications for the hip replacement were congenital hip dysplasia, trauma, and osteonecrosis of the femoral head. Patients with rheumatoid arthritis were excluded from the study, as well as patients with infection. In the group with loosening, 18 of 26 prostheses were cemented and 10 had a metal-on-polyethylene bearing. In the group with stable prostheses, only 2 implants were cemented; 14 had a metal-on-metal bearing.

Sample collection

Fasting blood was collected into 0.129 M trisodium citrate anticoagulant (9 parts of patient's blood to 1 part of citrate) in an ice water bath. The samples were centrifuged for 10 min at 3 000 r.p.m., then the plasma was collected and centrifuged at 11 000 r.p.m. for 10 min. Both centrifugations were performed at 4°C. The plasma was aliquoted and stored at –80°C.

PDGF-BB

PDGF-BB was determined by enzyme immunoassay (Quantikine Human PDGF-BB Immunoassay, R&D Systems, Minneapolis, MN, USA). The analysis was performed in duplicate for each patient sample. The wells were coated with a recombinant human PDGF Rβ/Fc chimera, which bound PGDF-BB 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 less than 8.4 pg/mL.

Latent TGF-ß1

Immediately prior to assay, latent TGF-β1 in plasma was activated by the addition of 1N HCl for 15 min, followed by neutralization with 1N NaOH. TGF-β1 was then measured by enzyme-linked immunosorbent assay (Bender Med Systems, Vienna, Austria). Each patient sample was analyzed in duplicate. The wells were coated with an anti-human TGF-β1 antibody, which bound TGF-β1 present in the samples or standards. A monoclonal mouse anti-TGF-β1 antibody bound to

any TGF-β1 captured by the first antibody. An anti-mouse IgG-biotin conjugate was then used to bind to the monoclonal anti-TGF-β1 antibody. Streptavidin-conjugated horseradish peroxidase (HRP) was used to bind to the biotinylated anti-mouse IgG, and the HRP was then detected by color change in its chromogenic substrate. The minimum detectable dose was 1.9 pg/mL.

Active TGF-ß1

Active TGF-β1 was determined directly in the plasma samples as above, but without activating them with HCl. Each patient sample was analyzed in duplicate.

Latent TGF-ß2

Immediately prior to assay, latent TGF-β2 in plasma was activated by the addition of 1N HCl for 15 minutes, followed by neutralization with 1N NaOH. TGF-β2 was then measured by enzyme-linked immunosorbent assay (Bender Med Systems, Vienna, Austria). Analysis of each patient sample was performed in duplicate. The wells were coated with an anti-human TGF-β2 antibody, which bound TGF-β2 present in the samples or standards. A monoclonal mouse anti-TGF-β2 antibody conjugated to biotin bound to any TGF-β2 captured by the first antibody. Streptavidin-conjugated horseradish peroxidase (HRP) was used to bind to the biotinylated anti-TGF-β2, and the HRP was detected by color change of its substrate. The minimum detectable dose was 10 pg/mL.

Active TGF-ß2

Active TGF-β2 was determined directly in the plasma samples as above, but without activating them with HCl. Each patient sample was analyzed in duplicate.

Statistics

The results are reported as the arithmetic mean (SE). Statistical analysis of the effects of the prosthesis failure or success was done by analysis of variance (ANOVA), and Bonferroni's multiple comparison test was applied to detect specific differences between groups. Correlations between laboratory tests and age were researched by the Spearman test. The level for significance was set at $p < 0.05$.

Results

PDGF-BB

The patients with prosthesis loosening had plasma PDGF-BB levels that were lower than those of the presurgery subjects (loosened prosthesis: 40 (8.4) pg/mL vs. presurgery: 90 (16) pg/mL; $p = 0.01$). There were no statistically significant differences between plasma PDGF-BB levels in patients with loosened or stable prostheses (53 (14) pg/mL), nor between levels in patients with stable prostheses and presurgery patients.

Latent TGF-ß1

There was no difference in latent TGF-β1 levels between patients with loosened (5789 (414) pg/mL) and stable prostheses (5951 (353) pg/mL), nor between those with loosened prostheses and presurgery patients (4804 (287) pg/mL). The patients with stable prostheses had significantly higher levels of latent TGF-β1 than presurgery patients ($p = 0.03$). No correlation between latent TGF-β1 and PDGF-BB levels was found in any group.

Active TGF-ß1

Active TGF-β1 levels in all groups were very low and near the detection limit. The patients with loosened prostheses had 40 (12) pg/mL of active TGF-β1, the patients with stable prostheses had 27 (6.5) pg/mL, and the presurgery group had 26 (8.5) pg/mL. There were no significant differences between groups. No correlation between active TGF-β1 and PDGF-BB levels, or between active and latent TGF-β1 levels, was found in any group.

Latent TGF-ß2

The patients with loosened prostheses had lower levels of latent TGF-β2 than the patients with stable implants (loosened prosthesis: 657 (168) pg/mL vs. stable prosthesis: 1579 (313) pg/mL; $p = 0.005$), but not lower than the presurgery individuals (395 (161) pg/mL; $p = 0.4$). The patients with stable prostheses had significantly higher levels than the presurgery patients ($p = 0.0002$). No correlation between latent TGF-β2 and PDGF-BB levels, or between latent TGF-β2 and active or latent TGF-β1 levels, was found in any group.

Active TGF-ß2

Active TGF-β2 levels in all groups were very low and near the detection limit. The patients with loosened prostheses had 50 (35) pg/mL of active TGF-β2, the patients with stable prostheses had 10 (0.05) pg/mL, and the pre-surgery group had 11 (0.9) pg/mL. There were no significant differences between groups. There was no correlation in any group between level of active TGF-β2 and levels of the other growth factors.

Discussion

We compared patients with loosened hip prostheses with patients who had stable prostheses in order to investigate the role of some growth factors in implant failure, and with patients who were candidates for total hip replacement in order to evaluate the effect of the prosthetic implant itself. The three subsets of patients were similar regarding gender and underlying illness. The patients with loosened prostheses were older than the patients with stable implants and the presurgery patients. However, the parameters analyzed were not correlated with age; nor were they affected by gender or by underlying illness. Presurgery patients were chosen as the reference group instead of healthy subjects, as osteoarthrosis, which is the most common cause of hip replacement, causes variations in the cytokines of cartilage (Moos et al. 1999), which may influence systemic levels. In addition, osteoarthritis may augment specific cellular activation, subsequently affecting local inflammatory responses to implant wear particles (Berry et al. 2002).

We were unable to assert whether the plasma levels of growth factors were affected by type of prosthesis, because the number of patients examined was too low. However, it can be noted that most loosened prostheses were cemented, whereas most stable prostheses had no cement. The highest number of cemented prostheses among the loosened ones could be determined also by the longer follow-up, since cementless prostheses have been used for fewer years. Finally, in the group of failed prostheses we found no significant variations in plasma levels of growth factors among the patients with different bearing surfaces, which was perhaps due to the low number of metal-on-metal hip replacements. However, other authors have found lower levels of TGF-β in periprosthetic tissues from failed metal-on-metal hip replacements than in tissues surrounding failed metal-polyethylene hip prostheses (Campbell et al. 2002).

When prosthetic materials undergo a wear process, an inflammatory reaction is induced. The cellular events at the interface between implant and host tissues have a potential role in the etiology of local bone resorption. Increased serum levels of TGF-β1 and IGF-I have been demonstrated in the third or fourth week of callus healing (Kaspar et al. 2003). Platelet-derived growth factor mRNA was found in macrophages and fibroblasts of membranous tissue obtained from the cement-bone interface of polyethylene acetabular components that had been revised for aseptic loosening (Jiranek et al. 1993). Thus, it has been hypothesized that PDGF causes hyperplasia of the connective tissue around the prosthesis.

In the present study, we analyzed growth factor concentrations in plasma, in order to evaluate patients with stable prostheses too. Previously, we have found no differences in plasma levels of PDGF-AB between patients with prosthesis loosening and presurgery patients, and between patients with prosthesis loosening and patients with fixed implants (Cenni et al. 2003). In contrast, PDGF-BB plasmatic levels were lower in the prosthesis-bearing patients than in presurgery subjects. The discrepancy between plasma PDGF-AB and PDGF-BB levels may be related to the lower biological activity of PDGF-AB. Higher levels of PDGF have been found in periprosthetic tissues (Xu et al. 1998). The discrepancy between tissue and plasma levels may be explained by the hypothesis that PDGF released into the periprosthetic microenvironment binds to cell receptors, or is degraded by activated proteases and does not enter the bloodstream.

Osteoblasts are an important source of TGF-β1 and TGF-β2. Both latent and activated forms of TGF-β1 and TGF-β2 were tested, because latent TGF may be activated by macrophages present in periprosthetic tissues, if stimulated by γ-interferon or IgG (Bonewald 1996). Increased expression of TGF-β1 and TGF-β2 has been shown in the pseudocapsula and in cells from hip aseptic loosening, compared with knee synovial membranes (Kont-

tinen et al. 1997). The patients with prosthetic loosening did not have significantly higher plasma levels of latent TGF-β1 than the presurgery subjects, and TGF-β2 levels were only slightly higher than in presurgery subjects, and significantly lower than in the patients with stable prostheses. The patients with fixed prostheses had significantly higher levels of latent TGF-β1 and TGF-β2 than presurgery subjects. The TGF-β2 results are similar to those we have measured for serum type I procollagen pro-peptide in loosened and fixed prostheses: in loosened prostheses they were similar to presurgery levels, but they were raised significantly in stable implants (unpublished observations).

Takagi et al. (2001) showed that in prosthesis loosening, the activation of monocyte/macrophages caused an accelerated bone remodeling, which resulted in the formation of poor-quality bone favoring osteolysis. The high bone turnover in periprosthetic tissues may explain the small increase in TGF-β1 and TGF-β2 observed in the loosened prostheses, while in stable ones bone remodeling results in the formation of a good-quality bone, and the release of TGF-β1 and TGF-β2 was higher. Differences were demonstrated only in the latent isoforms of TGF-β1 and TGF-β2, while the levels of active isoforms were similar in the three groups of patients. The low plasma levels of the active isoforms may be the result of low pH in the microenvironment of the prosthesis, which activates TGF.

Thus, it may be supposed that the prosthetic implant itself causes a local increase in PDGF-BB, TGF-β1 and TGF-β2, which are released by osteoblasts and other cells in the microenvironment. The local increase in PDGF-BB does not cause an increase in plasma levels, probably as a result of local consumption or degradation. In stable implants, the consumption of PDGF-BB is lower, while TGF-β1 and TGF-β2 levels increase during bone formation. In loosening, PDGF-BB consumption is higher and causes a significant reduction in plasma levels in comparison to presurgery; the formation of a poor-quality bone may be related to the scarce increase in TGF-β1 and TGF-β2.

We conclude that in patients with hip prosthesis loosening, a decrease in latent TGF-β2 can be shown relative to patients with stable prostheses, and a reduction in plasma PDGF-BB levels can be detected relative to the levels seen in presurgery subjects. In stable prostheses, a significant increase in latent TGF-β1 and TGF-β2 was seen relative to corresponding levels in presurgery patients. In loosening, unlike fixed prostheses, a reduction in the bone-forming growth factors appears to play a role. Larger series of patients will be required in order to determine whether the monitoring of growth factors might be useful in the early diagnosis of loosening.

No competing interests declared.

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