Protein expression of MMP-13, uPA, and PAI-1 in pseudocapsular and interface tissue around implants of loose artificial hip joints and in osteoarthritis

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Abstract. Matrix metalloproteinase 13 (MMP-13), urokinase type plasminogen activator (uPA), and plasminogen activator inhibitor type-1 (PAI-1) have been reported to be involved in aseptic loosening of artificial hip joints. This study for the first time presents the protein levels of all of these factors in synovial-like interfaces between bone and prosthesis and in pseudocapsular tissues surrounding the artificial joint in patients with aseptic loosening (n=17) measured by ELISA. No differences were observed in the antigen expression of MMP-13, uPA, and PAI-1, comparing interface and pseudocapsular tissue. Also, no significant correlation between the protein expression of these factors and years from arthroplasty to revision or to type of fixation (cemented vs. cementless) was observed. As control, MMP-13, uPA, and PAI-1 antigen levels were also determined in the synovium of patients with osteoarthritis (n=10). Yet, the antigen levels of MMP-13, uPA, and PAI-1 in tissue specimens from patients with aseptic loosening of artificial hip joints were significantly higher compared to their expression in synovial capsular tissues obtained from patients with osteoarthritis. In conclusion, this study shows that elevated protein levels of uPA, PAI-1, and MMP-13 in periprosthetic pseudocapsular and interface tissues from patients after total hip replacement due to aseptic loosening seem not to be associated with the patient outcome.

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Abreviations: PAI-1, plasminogen activator inhibitor type-1; uPA, urokinase type plasminogen aktivator; MMP, matrix metalloproteinase; OA, osteoarthritis; THR, total hip replacement

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Introduction

Aseptic loosening is the most critical event in total hip arthroplasties. Still, despite intensive research, the biological mechanisms leading to local tissue reaction around total hip arthroplasties resulting in abnormal bone resorption have not yet been clarified. One of the mechanisms leading to granulomatosis is mediated by the wear of implant materials. This results into release of microscopic small implant particles and larger debris into the periprosthetic interface and pseudocapsular tissue. Histological analysis of these tissues revealed granulomatous reaction mediated by monocytes, macrophages, fibroblasts, and vascular endothelial cells (1-3). This histological appearance is easily distinguished from that of osteoarthritis; only scattered foci of inflammatory cells are found in the synovial membrane (4).

Proteolytic enzymes are considered to play an important role in extracellular matrix degradation, also in that of the periprosthetic connective tissue. Most cell types involved in the granulomatous reaction have the potential to produce matrix metalloproteases (MMPs) (5) and components of the urokinase system such as the serine protease urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor type-1 (PAI-1). MMPs and uPA are considered to be important proteolytic enzymes involved in the loosening of total hip arthroplasty (3). Studies from Takei *et al* demonstrated elevated mRNA expression levels of MMP-1, MMP-9, MMP-10, MMP-12, and MMP-13 in the synovium-like interface tissue of aseptic loosening of artificial joints. In particular, digestion of collagen by MMP-13 which is produced by osteoblastic cells appears to be the initial and critical step of the entire bone resorption process (6). MMP-13 is secreted as an inactive zymogen and activated by membrane-type-1-MMP (MT1-MMP) and/or urokinase type plasminogen activator (uPA) prior being able to degrade substrates in the extracellular matrix (7-10).

The pro-form of uPA, pro-uPA, is secreted by e.g. macrophages (11), fibroblasts (12), and synovial cells (13) and can

be activated by cathepsins B and L and the membrane-type serine protease MT-SP1 (14,15). uPA is bound to its cell surface-associated receptor uPAR and can efficiently activate the zymogen plasminogen to the protease plasmin. *Vice versa*, the focalized activation of plasmin may lead to the activation of collagenases like MMP-13 and, thus, to tissue destruction and loosening of the prosthesis (16-19). Proteolytic degradation is controlled by protease inhibitors such as PAI-1, an inhibitor of uPA that is produced by platelets, fibroblasts, macrophages, and endothelial cells (11,13,20-22). Hence, the activation of the uPA system represents the initial step in the generation of a proteolytic active moiety (23) which consequently can lead to the activation of MMPs, eventually resulting in the loosening of implants.

Most studies performed so far (5,16,24) have focused on investigation of the RNA expression pattern of proteases in tissues of loose total hip replacements. Considering a possible post-translational modification of MMP-13-RNA in periprosthetic tissues, an analysis of its expression pattern not only at the mRNA level, but also at the protein level is desirable. This study aimed at the analysis of the protein levels of uPA, PAI-1 and for the first time MMP-13 in periprosthetic tissues and to elucidate their role in periprosthetic loosening by correlating their protein expression profile to follow-up data of patients with loosening total hip arthroplasty.

Materials and methods

Patients and clinical data. Seventeen patients who underwent revision of total hip arthroplasty between June 1998 and February 2000 caused by aseptic loosening were included in this study. Six of the 17 patients were male, 11 female; mean age was 68.3±8.4 (range 56-83) years.

To avoid inadequate sampling in terms of heterogeneity, two different tissue samples were obtained from each patient (one from the inner site and one from the outer site of the capsule/interface). In 12 cases capsular, in 5 cases interface tissue was obtained. In 3 of 5 cases only one specimen was sampled, owing the fact that the interface tissue was too small to distinguish between the inner and outer site. Capsular tissues of joints were additionally obtained from patients with osteoarthritis in the hip or knee (n=10). Six of the 10 patients were male, 4 female; mean age was 62.2±14.8 years (range 38-84) years.

Preparation of tissue extracts and measurement of MMP-13, uPA, and PAI-1. Immediately after excision, the tissue samples were snap-frozen in liquid nitrogen and stored at -78˚C. Tissue extracts were prepared by pulverizing the frozen tissue, followed by addition of 1 ml detergent containing Tris-buffered saline (0.02 M Tris-HCl/pH 8.5, 0.125 M NaCl, 0.1% Triton X-100) and centrifugation at 100,000 x g (1 h, 4˚C) as described previously (25). The protein levels of uPA and PAI-1 were determined by certified ELISA tests (uPA: Imubind 894 and PAI-1: Imubind 821; both from American Diagnostica Inc., Greenwich, CT) and were expressed as ng per mg of tissue protein. Determination of MMP-13 levels were performed by a newly established ELISA (26). Briefly, mono- and polyclonal antibodies (BioCheck, Münster, Germany) against the catalytic site of recombinant collagenase-3 (MMP-13) were used in a sandwich-type ELISA format. The MMP-13-ELISA recognizes active as well as the non-active precursor form of the enzyme with sensitivity range of 0.5 ng/ml and a low degree of cross-reactivity (<3%) for all of the MMPs tested. MMP-13 content is expressed in ng of MMP-13 per mg of tissue protein.

Statistical analysis. Non-parametric methods were used in the statistical analyses as various parameters did not follow the normal distribution. Results were expressed as median values and comparisons between groups done using Wilcoxon's rank-sum test. The significance level was set to 0.05.

Results

Clinical samples and patient data. Twelve samples of pseudocapsule tissue and five samples of interface tissue were obtained intraoperatively from 17 patients hospitalized for aseptic hip loosening. Loosening of the total hip (cup and stem) was found in 5 cases, cup loosening alone in 12 cases. In 7 cases the stem/cup was fixed with cement (polymethyl methacrylate), in ten cases no cement was used. The average time from arthroplasty to revision was 10.4±3.9 (range 3-16) years. Clinical data of the patients are listed in Table I.

Protein levels of MMP-13, uPA, and PAI-1 in the interface and pseudocapsular tissue of patients after total hip replacement (THR). Protein levels of MMP-13 in 17 cases of periprosthetic weakening were between 0.1-12.3 ng/mg with a median value of 0.4 ng/mg protein. MMP-13 was detected in 13 tissue specimens. In four tissue specimens MMP-13 protein levels were below the detection limit of 0.1 ng MMP-13/mg protein. In these cases, the amount of MMP-13 protein was set to 0.1 ng/mg for statistical reasons. There was no difference in the expression rate of MMP-13 between pseudocapsule and interface tissue. Protein levels of uPA were between 0.2-2.75 ng/mg protein with a median value of 1.3 ng/mg. There was no difference between pseudocapsule and interface tissue regarding expression of uPA. Protein levels of PAI-1 were between 8.65-433.0 ng/mg tissue protein with a median of 89.2 ng/mg protein. There was no difference between pseudocapsule and interface tissue regarding expression of PAI-1.

Expression level correlates to site of tissue. The tissue samples obtained from the inner and the outer side of the synovial like-membrane showed no difference in antigen expression of MMP-13 and uPA. Protease expression turned out to be constant in the pseudocapsular tissue whereas non-significant variation in PAI-1 expression was seen in some cases (Table I).

Protein expression level of MMP-13, uPA and, PAI-1 does not correlate to length of time from arthroplasty to revision and to the type of stem and cup fixation. Although the number of the examined cases was too small for extensive statistical analyses, there was no indication that cement fixation of the joint had a specific effect on the expression levels of MMP-13, uPA, or PAI-1. None of these proteolytic factors showed any significant difference in antigen expression with respect to the fixation

Table I. Clinical data of the patients with artificial hip joints.

Case	A/G	Site	Procedure	Years	Fixation	MMP-13		uPA		PAI-1	
						Out	In	Out	In	Out	In
1	76/F	Cap	THR	15	C^+	0.1	0.7	2.2	2.5	33.4	34.1
2	70/F	Cap	CR	8	C^-	0.1	0.1	0.7	0.6	14.0	11.2
3	62/M	Cap	THR	5	C^-	3.0	5.1	0.5	1.3	486.8	243.8
4	70/F	Cap	THR	6	C^-	1.9	0.2	0.3	1.2	8.3	9.0
5	80/F	Cap	CR	12	C^-	11.4	13.2	1.9	1.3	102.5	38.3
6	61/M	Cap	THR	14	C^+	0.1	0.1	2.4	3.1	92.8	55.7
7	70/F	Cap	THR	11	C^+	0.1	0.1	0.9	0.6	100.9	77.4
8	74/F	Int	CR	13	C^+	8.3		2.2		150.2	
9	61/F	Cap	CR	15	C^-	0.1	0.3	2.1	1.0	95.7	109.7
10	78/F	Cap	CR	3	C^-	0.1	0.1	1.8	1.1	303.1	562.8
11	76/M	Int	CR	6	C^-	0.3		0.3		104.3	
12	71/F	Int	CR	16	C^+	0.4	2.5	1.2	1.6	145.1	347.2
13	56/F	Cap	CR	8	C^-	9.6	5.3	1.6	2.5	57.7	47.1
14	83/F	Int	CR	13	C^-	2.0		0.5		188.2	
15	77/M	Cap	CR	13	C^-	1.2	0.7	1.4	1.0	48.5	64.8
16	78/M	Int	CR	10	C^-	0.2	0.2	0.2	0.2	16.8	13.3
17	86/M	Cap	CR	9	C^+	0.2	0.4	1.0	1.0	147.0	141.7

Protein levels of MMP-13, uPA, and PAI-1 were measured in the interface and pseudocapsular tissue of the patients. A/G, age and gender of the patient; F, female; M, male; Cap, capsule (tissue was obtained from the inner (in) or outer side (out) of the pseuducapsule surface); Int, interface tissue; THR, total hip replacement due to loosening; CR, artificial cup replacement due to loosening; years, years from arthroplasty to revision; C^+, fixation of the stem/cup using cement (polymethyl methacrylate); C^-, fixation of the stem/cup without using cement; MMP-13, uPA, and PAI-1, tissue level of the respective analyte as measured by ELISA, expressed as ng analyte/mg tissue protein.

procedure. There was no correlation between MMP-13, uPA, or PAI-1 expression levels and the length of time from arthroplasty to revision.

Protein expression levels of MMP-13, uPA, and PAI-1 in the capsular tissue of patients with osteoarthritis. Capsule tissues were also sampled from patients with osteoarthritis (n=10) undergoing total hip replacement (Table II). MMP-13 was between 0.1-3.6 ng/mg tissue protein with a median of 0.1 ng/mg total protein. In 6 cases, MMP-13 levels were below detection limit of 0.1 ng MMP-13/mg protein. Levels of uPA were between 0.1-1.3 ng/mg tissue protein with a median of 0.4 ng/mg tissue protein. Levels of PAI-1 were between 7.4-200.8 ng/mg tissue protein with a median of 21.7 ng/mg protein.

Comparison of MMP-13, uPA, and PAI-1 protein levels in patients with aseptic loosening and osteoarthritis. The median pseudocapsular tissue levels of MMP-13 (p=0.048), uPA (p=0.005), and PAI-1 (p=0.031) from patients with total hip replacement due to aseptic loosening was statistically significantly elevated over those determined in patients with osteoarthritis (Fig. 1).

Table II. Clinical data of patients with osteoarthritis.

Case	A/G	Site	MMP-13	uPA	PAI-1
1	69/M	Capsule hip	0.1	0.3	58.4
2	81/M	Capsule hip	0.1	1.0	208.4
3	69/F	Capsule knee	0.1	0.7	8.6
4	49/M	Capsule hip	0.1	0.6	8.4
5	51/F	Capsule hip	0.1	1.3	171.6
6	38/F	Capsule hip	3.6	0.2	19.1
7	51/F	Capsule hip	1.3	0.1	7.4
8	61/M	Capsule hip	0.6	0.3	19.8
9	84/M	Capsule hip	0.5	0.4	23.5
10	69/M	Capsule hip	0.1	1.0	29.9

Tissue specimens were sampled from the capsule of the hip or knee. A/G, age and gender of the patient; F, female; M, male; MMP-13, uPA and PAI-1, tissue level of the respective analyte as measured by ELISA expressed as ng analyte/mg tissue protein.

Discussion

In this study, the protein expression levels of uPA, PAI-1 and for the first time MMP-13 were analyzed in pseudocapsular and interface tissue of loose artificial hip joints and correlated to patient outcome. No difference in the expression levels of MMP-13, uPA, and PAI-1 between pseudocapsular and interface tissue was detected. This is in agreement with immuno-

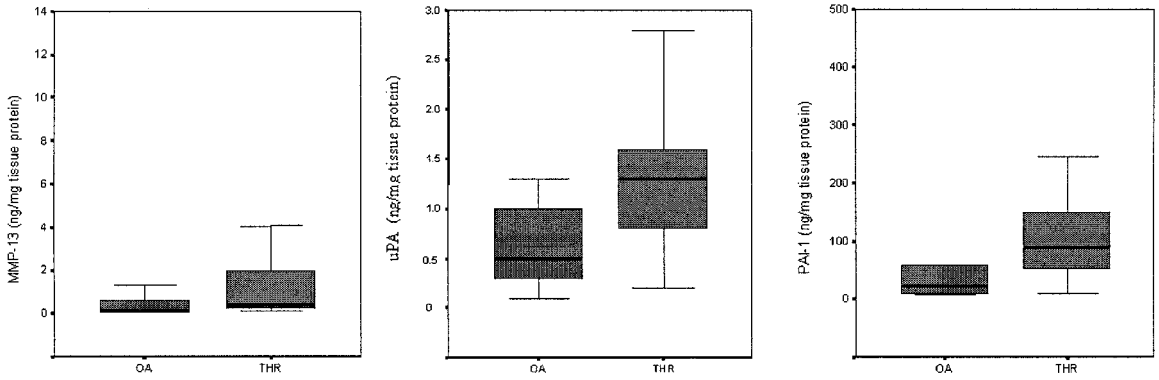

Figure 1. Comparison of the median tissue levels of MMP-13, uPA, and PAI-1 in pseudocapsular tissue from patients with total hip replacement (THP) (n=17) due to loosening and patients with osteoarthritis (OA) (n=10). All of the three analytes were expressed in significantly higher amounts in patients with aseptic loosening of the hip compared with patients with osteoarthritis [uPA (p=0.005), PAI-1 (p=0.031), MMP-13 (p=0.048)]. Boxes show the median as a line and the 25th and 75th percentiles. Bars, the 5th and 95th percentiles of the values of each group.

histochemical findings in pseudocapsular and interface tissue of loose artificial hip joints regarding collagenase (27), uPA, and PAI-1 (28). In contrast to the small tissue sample size of interface tissue obtained after surgery, pseudocapsular tissue can be sampled at >10-fold in volume compared to interface tissue. To investigate protein expression differences between the inner and the outer site of the pseudocapsular tissue, and to avoid inadequate sampling in terms of heterogeneity, two samples were obtained from each patient. In general, the expression rates were not different when tested in tissue of different locations. Ishiguro *et al* (24) observed different mRNA expression of MMP-1, MMP-2, MMP-3, MMP-9 in different samples from the same interface tissue. Furthermore, Imai *et al* and Takei *et al* reported elevated MMP-13 mRNA expression levels in the synovium-like interface tissues between bone and prosthesis of aseptic loose artificial hip joints (5,16). However, possible post-translational modification of MMP-13 were not taken into account and analyzed in these reports.

No correlation between the protein expression levels of the different proteins and the type of stem or cup fixation were found in this study. Both types of fixation - cementless and with cement - are commonly used in clinical practice today (29-31). This indicates that once a cellular host reaction occurs in the periprosthetic tissues and causes loosening, e.g. caused be the wear of implants, the expression of MMP-13, uPA, and PAI-1 is induced, regardless of the type of fixation, which is in accordance with findings by Takagi (3). There was also no correlation between time to revision from arthroplasty and protein expression levels of the factors in question. This finding is worth mentioning as both MMP-13 and uPA are supposed to play a crucial role during tissue destruction and bone resorption (18,32) eventually leading to aseptic loosening after arthroplasty. In addition, locally produced MMP-13 released into joint fluid can penetrate the interface between bone and prosthesis caused by high fluid pressure within the closed pseudojoint cavity and pumping effect during gait (17,33). Proteolytic action of MMP-13 will lead to extracellular matrix degradation and than to periprosthetic weakening (5,32).

It seems reasonable to compare data sets from patients with osteoarthritis and loose artificial hip joints even though osteoarthritic capsule tissue is different from normal tissue due to the presence of scattered foci of inflammatory cells (4). It has been reported that there is no difference in antigen levels of uPA and PAI-1 in synovium from patients with primary arthrosis and that from patients during knee arthroscopy due to meniscal tears (28). Investigations by Busso *et al* demonstrated variable amounts of uPA activity in osteoarthritis, associated with increased levels of uPA antigen and its corresponding mRNA, localized over the synovial proliferative lining areas (34). Saxne and co-workers suggested that uPA and PAI-1 may be less important in osteoarthritis because protein concentrations of these factors in synovial fluid from patients with osteoarthritis were not increased (35). mRNA levels of MMP-13 in osteoarthritis compared to rheumatoid arthritis revealed MMP-13 overexpression in the synovial fluid of rheumatoid arthritis patients and only to a minimum extent, if at all, in synovial fluid of osteoarthritis patients (36).

In this study, the protein expression levels of MMP-13, uPA, and PAI-1 were significantly higher in loose artificial hip joints compared to their expression levels in synovial capsular tissues obtained from patients with osteoarthritis indicating a potential role of these proteins in the loosening process. Similar findings have been reported for pseudocapsular and interface tissues from patients after total hip replacement revision for aseptic loosening (3). MMP-13 levels in control tissues from patients with medial neck fracture of the hip and from patients who underwent knee arthroscopy due to meniscus injury were significantly lower (3). Different results were obtained by immunoblot analysis of joint fluid samples obtained from patients with osteoarthritis and total hip replacement where moderate immunoreactivity to pro-MMP-13 was detected in both tissue specimens (17).

Taken together, the present study demonstrates elevated protein levels of uPA, PAI-1 and for the first time of MMP-13, in periprosthetic pseudocapsular and interface tissues from patients after total hip replacement due to loosening. Although the factors are not associated with patient outcome it seems due to their increased protein expression reasonable to speculate that these enzymes are involved in biological processes mediating loosening of implants in total hip arthroplasty.

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