

High levels of substance P and CGRP in pseudo-synovial fluid from patients with aseptic loosening of their hip prosthesis

Yebin Qian, Bing-Fang Zeng, Xian-Long Zhang, and Yao Jiang

Department of Orthopedic Surgery, the Sixth People's Hospital, College of Medicine, Shanghai Jiaotong University, Shanghai, China
Correspondance BZ: zengbingfang@yahoo.com.cn
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Background Aseptic loosening is the most important complication after total hip arthroplasty (THA). The nervous system has been implicated in the etiology and pathogenesis of joint diseases.

Methods We compared levels of substance P (SP) and calcitonin gene-related peptide (CGRP) in pseudo-synovial fluid from patients with aseptic loosening after THA with those in synovial fluid from patients undergoing primary THA for osteoarthritis, who served as controls. Levels of SP and CGRP were measured using an enzyme immunoassay.

Results We found that SP and CGRP levels were significantly higher in the pseudosynovial fluid of loose artificial joints than in the synovial fluid of controls.

Interpretation SP and CGRP may have a role in aseptic loosening.

The most frequent mode of failure after arthroplasty is aseptic loosening due to periprosthetic osteolysis. The pathogenesis of prosthetic loosening is still unknown. Wear debris has been extensively studied as a cause of osteolysis. Prior work has shown a relationship between the generation of particles and aseptic loosening. Cytokines that have been demonstrated in the periprosthetic tissues include tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-6, and receptor activator of nuclear factor kappa B ligand (RANKL) (Haynes et al. 2001). These factors are involved in complex signaling pathways for osteoclast activation.

Synovial membrane-like interface tissue develops between implants and periprosthetic reactive

cancellous bone-like cortex. This fibrous tissue mainly consists of fibroblasts, macrophages, and a vascular component. Pseudosynovial fluid probably contributes to the development of the interface tissue. As a result of fluid pressure waves during cyclic loading, the fluid gradually penetrates between the implant and the host bone. Pseudosynovial fluid has already been recognized to act as a liquid transport medium for particulate ultra-high molecular weight polyethylene debris (Robertsson et al. 1997). The pseudosynovial fluid contains cytokines such as IL-1, IL-6, TNF, and RANKL (Nivbrant et al. 1999, Mandelin et al. 2005). It may thus constitute a transport vehicle for potent biologically active osteoclastogenic factors.

Pseudosynovial fluid from a loosened total hip prosthesis can induce osteoclast formation (Mandelin et al. 2005). The nervous system has been implicated in the etiology and pathogenesis of joint diseases. SP and calcitonin gene-related peptide (CGRP) are sensory neuropeptides. CGRP has been found to increase IL-6 and IL-8 secretion in RA fibroblasts and SP has been found to stimulate IL-8 secretion in OA fibroblasts (Raap et al. 2000). It is widely accepted that SP is a proinflammatory neurotransmitter. For example, SP stimulates expression of IL-1, TNF (Lotz et al. 1988), IL-2 (Rameshwar et al. 1993), and nuclear factor kappa B (Lieb et al. 1997) in various cell types. This indicates that it is a potent proinflammatory agent. There is only limited information regarding the role of SP and CGRP in aseptic loosening. SP- and CGRP-immunoreactive nerve fibers have been detected in the pseudocapsular tissues (Niissalo

et al. 2002) and interface membranes (Ahmed et al. 1998) of aseptically loosened hip prostheses. In this study, we investigated the levels of SP and CGRP in the pseudosynovial fluid from patients with aseptic loosening after total hip arthroplasty (THA), and compared them with the levels in synovial fluid of patients undergoing primary THA for osteoarthritis.

Patients and methods

Samples of pseudosynovial fluid were collected from 10 patients (6 women) undergoing revision operation due to aseptic loosening of THA. All hips had radiographic osteolysis and had a loose implant at revision. The indication for primary THA had been osteoarthritis in all cases. There were no clinical or laboratory signs of infection in any patient. The mean time from primary arthroplasty to revision was 10 (3–19) years. The type of prostheses was Charnley and all of them were cemented. The mean age at revision was 70 (62–81) years.

The fluid samples were aspirated with a syringe and needle before incision of the joint capsule. Control synovial fluid samples were collected by the same method from 12 patients (mean age 58 (50–72) years, 7 women) undergoing primary THA for osteoarthritis of the hip. All patients had advanced disease radiographically. Patients with rheumatoid arthritis or other forms of inflammatory arthritis were excluded from serological examination and clinical features.

All samples were immediately centrifuged at 3,000 rpm for 15 min to remove cellular debris, and the supernatants were frozen and stored at -80°C until analysis. The concentrations of SP in synovial fluid and pseudosynovial fluid were measured using enzyme immunoassay (EIA) kits (Cayman Chemical, Ann Arbor, MI) in accordance with the directions of the supplier. The detection limit was 8 pg/mL. CGRP was measured using EIA kits (SPIbio, city, France) in accordance with the instructions of the manufacturer. The detection limit was 7.81 pg/mL.

The study was conducted with the informed consent of patients and was approved by the local Ethics Committee (2005-12-03).

Statistics

All values are expressed as mean (SD). The Mann-Whitney U-test was used to compare values in patients with prosthesis loosening to those in patients with OA. We considered p-values of < 0.05 to be statistically significant.

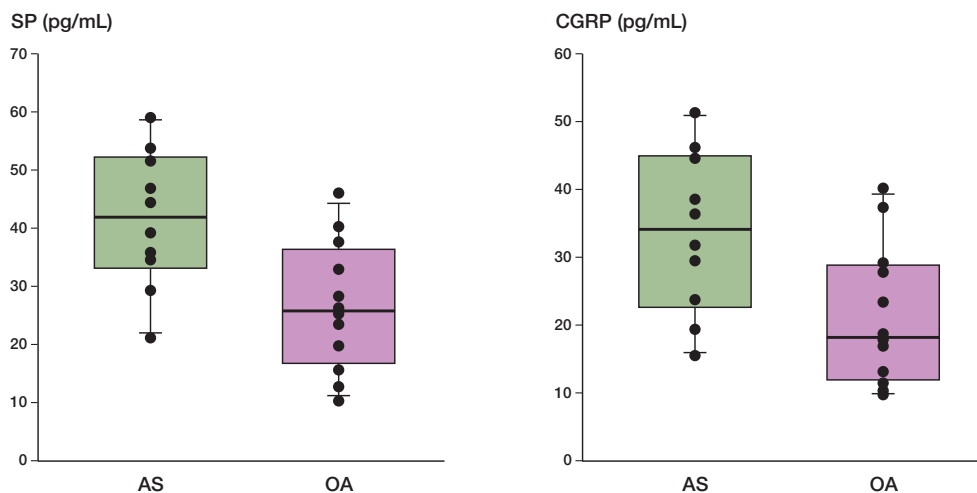
Results

The mean concentration of SP was 42 (10) pg/mL in pseudosynovial fluid from patients with aseptic loosening and 27 (12) pg/mL in synovial fluid from patients with OA who were undergoing primary THA ($p = 0.01$) (Figure 1A).

The mean concentration of CGRP was 33 (10) pg/mL in pseudosynovial fluid from patients with aseptic loosening and 21 (12) pg/mL in synovial fluid from patients with OA undergoing primary THA ($p = 0.02$) (Figure 2B).

Discussion

Relevant studies have focused mainly on the role of cellular mediators in aseptic loosening of prostheses and the presence of sensory neuropeptides in the pseudosynovial fluid has not been investigated. However, the nervous system has been implicated in the etiology and pathogenesis of joint diseases. The involvement of neurogenic inflammation in adjuvant-induced experimental arthritis has been demonstrated in rats (Levine et al. 1984). SP- and CGRP-immunopositive nerve fibers in the joints of patients with painful osteoarthritis of the hip have been found in the soft tissue of the fossa acetabuli, as well as in the synovial layer of the hip joint capsule (Saxler et al. 2007). Joints with severe arthritis have a dense innervation of SP-containing sensory neurons and a higher SP content than joints that develop mild arthritis (Weidler et al. 2005). Infusion of SP into the knee exacerbates the severity of experimental arthritis (Levine et al. 1984, 1985). There are increased levels of SP in the synovial fluid and serum of patients with RA (Marshall et al. 1990, Menkes et al. 1993). These findings support the possibility of SP and CGRP having a role in bone lesion development. In this study, we found that there were higher levels of SP and CGRP in the pseudosynovial



Box plots showing SP (left) and CGRP (right) concentrations in pseudosynovial fluid from patients with aseptic loosening (AS) after THA, and in synovial fluid from patients with OA undergoing primary THA. The box plots show the tenth and ninetieth percentile, the twenty-fifth and seventy-fifth percentile, and the median. The concentrations of SP and CGRP in pseudosynovial fluid were higher than in synovial fluid ($p = 0.01$ and $p = 0.02$).

fluid. SP- and CGRP-immunoreactive nerve fibers have been detected in the pseudocapsular tissues (Niissalo et al. 2002) and in the interface membranes (Ahmed et al. 1998) of aseptically loosened hip prostheses. SP- and CGRP-immunoreactive axons have been shown to be localized in bone (Bjurholm et al. 1991). In the pathogenesis of aseptic loosening, loose implants, wear particles, and cytokines may stimulate the surrounding nerves and promote the release of sensory neuropeptides.

SP and CGRP may be transported in the pseudosynovial fluid and act on the different cells they contact, such as fibroblasts, macrophages, lymphocytes, and osteoclasts. SP promotes osteoclastogenesis through both upregulation of RANKL expression and downregulation of OPG expression in synovial fibroblastic cells, and induction of the proliferation of synovial fibroblastic cells (Matayoshi, et al. 2005). SP has been found to stimulate the production of prostaglandin (PG) E₂ and RANKL in fibroblast-like cells of human dental pulp, and to promote bone resorption (Kojima et al. 2006). Fibroblasts, lymphocytes, and macrophages are equipped with SP receptors and SP has been shown to induce the release of IL-1, IL-6, and TNF from these cells (Yamaguchi et al. 2004). SP receptors have also been found on the plasma membrane and in the cytoplasm of osteoclasts (Goto et al. 1992). In an experiment using mouse calvaria, SP caused

increased bone resorption (Sherman et al. 1995). The addition of SP was also found to increase the bone resorption activity of cultured rabbit osteoclasts (Mori et al. 1999). These findings suggest that the neuropeptide SP is one of the risk factors in bone resorption, and that it may participate in the periprosthetic osteolysis. The role of CGRP is ambiguous. CGRP stimulates lymphocyte proliferation (Casini et al. 1989). There have been few reports documenting an effect of CGRP on the production of proinflammatory factors by macrophages. Feng et al. (1997) reported that CGRP reduces TNF production in response to lipopolysaccharide (LPS). According to Torii et al. (1997), IL-1 β production of macrophages in response to LPS diminishes in the presence of CGRP. However, Cuesta et al. (2002) reported an enhancement effect of CGRP on the basal secretion of IL-1 β , TNF, and IL-6 from human peripheral blood mononuclear cells. Yaraee et al. (2003) suggested that CGRP has the ability to induce production of TNF and IL-1 β . We found that the levels of CGRP in pseudosynovial fluid were increased in this study.

In conclusion, we have found elevated levels of SP and CGRP in the pseudosynovial fluid of patients with aseptic loosening. As SP and CGRP may be implicated in bone resorption and subsequent aseptic loosening of joint implants, these findings are certainly worthy of further study.

Contributions of authors

YQ designed the study and performed the research, collected and analyzed the data, and prepared the manuscript. BZ designed the study, analyzed the data, and also prepared the manuscript. XZ and YJ collected the data, and prepared the manuscript.

No competing interests declared.

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