Relationship of plasma and synovial fluid BMP-7 with disease severity in knee osteoarthritis patients: a pilot study

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Abstract The objective of this study was to investigate bone morphogenetic protein-7 (BMP-7) levels in both plasma and synovial fluid of patients with primary knee osteoarthritis (OA) and to determine their relationship to disease severity. Thirty-two patients with knee OA and 15 healthy subjects were enrolled in the study. Anteroposterior knee radiographs were taken to determine the disease severity of the affected knee. The radiographic grading of OA in the knee was performed using the Kellgren-Lawrence criteria. BMP-7 levels in the plasma and synovial fluid were measured using enzyme-linked immunosorbent assay. The mean plasma

BMP-7 concentration of the knee OA patients was significantly higher compared with that of healthy controls (12.1 ± 1.6 vs 3.5 ± 0.9 pg/ml, $P=0.001$). Although BMP-7 levels in plasma were higher with respect to paired synovial fluid samples, the difference was not statistically significant (12.1 ± 1.6 vs 10.5 ± 2.2 pg/ml, $P=0.3$). Subsequent analysis showed that plasma BMP-7 levels significantly correlated with disease severity ($r=0.77$, $P<0.001$). Furthermore, the synovial fluid levels of BMP-7 also correlated with disease severity ($r=0.60$, $P<0.001$). In addition, plasma BMP-7 levels showed a positive correlation with synovial fluid BMP-7 levels ($r=0.71$, $P<0.001$). Overexpression of BMP-7 in plasma and synovial fluid is related to progressive joint damage in knee OA. These findings suggest that BMP-7 might serve as a biochemical parameter for determining disease severity in primary knee OA and could play a potential role in cartilage protection and repair of OA.

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Introduction

Osteoarthritis (OA) is a strongly age-related joint disorder that is defined as a gradual loss of articular cartilage, combined with thickening of the subchondral bone, bony outgrowth at joint margins, and mild, chronic nonspecific synovial inflammation [8]. The most common parts in the knee to be affected are the medial tibiofemoral and lateral patellofemoral compartments. The clinical features of OA include pain, short-lasting stiffness, cracking of joints, joint swelling, and limited range of motion. Diagnosis of OA is dependent upon history and physical examination in conjunction with radiographs demonstrating joint space narrowing, osteophyte formation, and subchondral bone sclerosis. Although clinical research on OA has been

extensively investigated, the exact cause of this disease remains largely unclear.

Bone morphogenetic proteins (BMPs) have long been known to elicit wide-ranging biological activities, including the development, homeostasis, and repair of different tissues [10]. BMP-7, also known as osteogenic protein-1 (OP-1), is one of 15 currently known BMPs, which belong to the transforming growth factor- β (TGF- β) superfamily. BMPs are highly conserved across animal species, and mature human and mouse BMP-7 share 98% amino acid sequence identity [17]. BMP-7 was originally identified as a regulator of cartilage and bone induction activity [25]. Importantly, BMP-7 has been implicated in the stimulation of cartilage repair and the maintenance of articular cartilage integrity [24]. Several studies have shown regeneration of articular cartilage defects by BMP-7 in various in vitro experiments and large animal models [13, 20, 21]. Both pro-anabolic and anti-catabolic properties of BMP-7 suggest considerable promise for BMP-7 as a beneficial agent in cartilage repair [3]. Moreover, genomic approaches have concentrated on development of novel diagnostic and therapeutic strategies in repairing the damaged cartilage [1]. The role of BMP-7 in the process of pathological cartilage degeneration could raise the possibility that BMP-7 may play a role in the pathogenesis of knee osteoarthritis. Although plasma and/or synovial fluid levels of several cytokines have been examined in patients with knee OA, there have been no detailed studies on circulating and synovial fluid levels of BMP-7 in various clinical stages of primary knee OA [2, 6, 14, 19, 22].

In this study, we postulated that elevated BMP-7 levels in plasma and synovial fluid might be associated with the severity of clinical outcomes in knee OA patients. Therefore, the objective of this study was to analyse the concentrations of BMP-7 in both plasma and synovial fluid of patients with primary knee osteoarthritis, and determine the possible correlations of plasma and synovial fluid BMP-7 with the radiographic grading of knee OA.

Materials and methods

Patients

Ethical approval of this study was obtained from the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was received from the patients and healthy volunteers prior to their participation in the study.

Thirty-two patients aged 53 to 83 years diagnosed with primary knee osteoarthritis (28 females and four males;

mean age 70.5 ± 1.3 years) according to the criteria of the American College of Rheumatology and 15 normal healthy individuals (ten female and five male; mean age 65.5 ± 0.6 years) were enrolled in the study. All patients underwent total knee arthroplasty at our hospital between August 2007 and March 2008. Participants were excluded on the basis of having arthropathy due to gout, pseudogout rheumatoid arthritis, systemic lupus erythematosus, psoriasis, haemochromatosis, previous knee injury, or previous joint infection. Patients with any systemic inflammatory or autoimmune disorders, any types of malignant or chronic illness were not included in this study.

The severity of the disease was determined using weight-bearing anteroposterior radiographs of the affected knee. Knee radiographs were evaluated according to the Kellgren and Lawrence classification [15]: grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour; grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour. The grading scale used for analysis was the higher of the two knees.

Laboratory methods

One to two millilitres of synovial fluid was aspirated from the affected knee using sterile knee puncture just prior to surgery (total knee arthroplasty), centrifuged to remove cells and joint debris, and stored immediately at -80°C until the day of measurement. Blood samples were also collected from the patients one day before surgery, centrifuged to remove cells and debris, and stored at -80°C until used. Double-blind quantitative detection of BMP-7 in plasma and synovial fluid was performed using commercial enzyme-linked immunosorbent assay (ELISA) (Quantikine, R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol. Briefly, standards of recombinant human BMP-7, plasma, and synovial fluid samples were added to 96-well microtiter plates precoated with mouse monoclonal antibody against human BMP-7 and incubated for two hours at room temperature. The wells were then washed four times with washing buffer and incubated for two hours at room temperature with a horseradish peroxidase-conjugated monoclonal antibody against BMP-7. After four washes, substrate solution was added to each well, and the plate was incubated for 30 minutes at room temperature in the dark. Finally, the reaction was stopped with the stop solution, and then absorbance was measured at 450 nm using an automated microplate reader. Recombinant human BMP-7 was used to generate a standard curve.

Statistical analysis

Statistical analysis was carried out with the statistical package for social sciences (SPSS) software, version 16.0 for Windows (SPSS Inc., Chicago, USA). Comparisons between the groups were performed using one-way analysis of variance (ANOVA). Pearson's correlation coefficient was employed to determine the correlation among the concentration of BMP-7 in the plasma and synovial fluid and the disease severity. Data were expressed as a mean $\pm$ SEM. P values <0.05 were considered statistically significant.

Results

Plasma and synovial fluid BMP-7 levels of knee OA patients and plasma of controls are demonstrated in Fig. 1. OA patients had higher plasma BMP-7 concentrations compared to healthy controls (12.1 ± 1.6 vs 3.5 ± 0.9 pg/ml, $P=0.001$). Although the mean BMP-7 levels in plasma were higher than those in synovial fluid samples, the difference was not statistically significant (12.1 ± 1.6 vs 10.5 ± 2.2 pg/ml, $P=0.3$).

According to the Kellgren and Lawrence (KL) grading scale, eight patients were KL grade 2, whereas 12 patients were KL grade 3, and 12 patients were KL grade 4 osteoarthritis. The plasma and synovial fluid levels of BMP-7 were analysed and compared in relation to radiological KL grading of OA. The plasma BMP-7 levels from KL grade 2 were 4.1 ± 1.1 pg/ml, those from KL grade 3 were 8.0 ± 0.9 pg/ml, and those from KL grade 4 were 21.5 ± 2.4 pg/ml (Fig. 2). The results showed that plasma BMP-7 levels in KL grade 4 were significantly higher than those of KL grade 2 and 3 ($P<0.001$). Although the mean plasma levels of BMP-7 in KL grade 3 were greater than

Fig. 1 BMP-7 levels in plasma and synovial fluid of patients with OA and controls (* $P=0.001$)

Fig. 2 Plasma BMP-7 levels correlated with severity of OA ($r=0.77$, $P<0.001$)

those in KL grade 2, the difference was not statistically significant ($P=0.2$). In addition, the synovial fluid levels of BMP-7 from KL grade 2 were 2.6 ± 0.9 pg/ml, those from KL grade 3 were 5.6 ± 0.9 pg/ml, and those from KL grade 4 were 20.6 ± 4.6 pg/ml (Fig. 3). The data revealed that synovial fluid BMP-7 levels in KL grade 4 were significantly elevated compared with those of KL grades 2 and 3 ($P<0.002$). We further analysed the correlation between plasma and synovial fluid levels of BMP-7 and the severity of osteoarthritis. The plasma BMP-7 levels significantly correlated with the severity of disease ($r=0.77$, $P<0.001$) (Fig. 2). The synovial fluid levels of BMP-7 also correlated with disease severity ($r=0.60$, $P<0.001$) (Fig. 3). Interestingly, plasma BMP-7 levels showed a positive correlation with synovial fluid BMP-7 levels ($r=0.71$, $P<0.001$) (Fig. 4).

Discussion

BMPs have been implicated in biological activities, including the regulation of cell proliferation, apoptosis, differentiation and migration, embryonic development, and the maintenance of tissue homeostasis [11]. BMPs stimulate a complex cascade of events resulting in new bone formation and bone remodelling and play a critical role in ossification processes [12]. In particular, BMP-7 (or OP-1) is best known as a potent osteoinductive factor that has been shown to have promising potential as a cartilage anabolic factor because of its capabilities to induce matrix synthesis and promote cartilage repair [9]. Recent studies have demonstrated that BMP-7 is endogenously expressed in cartilage and functions as an anabolic factor for chondrocytes [7, 16]. Additionally, BMP-7 is localised in synovial fluid, synovium, ligament, tendon, and meniscus [23].

Fig. 3 Synovial fluid levels of BMP-7 correlated with severity of OA ($r=0.60$, $P<0.001$)

It has been shown that BMP-7 was identified in synovial fluid of patients with rheumatoid arthritis and with OA [2]. However, the relationship between BMP-7 levels in the plasma and synovial fluid and disease severity has never been specifically determined in patients with knee OA. To the best of our knowledge, no study dealing with correlation of BMP-7 levels in plasma and synovial fluid and severity of knee OA has been previously documented in the literature. This study is the first to show that BMP-7 was detected in both plasma and synovial fluid derived from patients with primary knee OA, and that BMP-7 positively correlated with the severity of OA.

This study showed a marked increase of BMP-7 levels in both plasma and synovial fluid of patients with knee osteoarthritis compared to the control plasma levels. Our findings suggest enhanced local and systemic production of

BMP-7 in primary knee osteoarthritis. There are two possible mechanisms to explain why BMP-7 levels in synovial fluid were elevated. First, high BMP-7 levels in synovial fluid are possibly caused by either the release of BMP-7 residing in extracellular matrix, or by increased production, or both. Second, synovial cells and chondrocytes in the local tissues (such as the synovial membrane and articular cartilage) could express endogenous BMP-7 in an autocrine/paracrine manner to increase endogenous BMP-7 in synovial fluid. In addition, BMP-7 might be derived from any of the other connective tissues of the synovial joint—meniscus, ligament, and tendon—since BMP-7 has been identified in all connective tissues of the joint [23].

It is intriguing to note that circulating BMP-7 was more pronounced in the OA patients than in the normal controls. Although bone is one of the main sources of BMP-7, other extraskeletal tissues may play a part in plasma BMP-7 elevation. The adult kidney is a possible major site of BMP-7 synthesis which might constantly release BMP-7 into the circulation of healthy individuals, while elevated values in patients with OA could reflect a release of BMP-7 from injured joints. The mechanism underlying circulating BMP-7 elevation in OA requires further investigation.

Recent studies have demonstrated that both BMP-7 protein and message were present in human adult articular cartilage [5]. Expression of BMP-7 mRNA was upregulated more than two-fold in OA cartilage compared with normal cartilage [5]. Furthermore, high levels of activated BMP-7 could be responsible for reparative response associated with early OA change [4]. These data suggest that endogenous BMP-7 may be involved in cartilage repair mechanisms of OA. Based on current evidence, it is tempting to speculate that the increased BMP-7 concentration in patients with OA reflects the level of intrinsic regenerative potential rather than a progression of joint cartilage destruction. More studies are needed to elucidate this observation.

In this study, the results indicate that the increased plasma and synovial fluid levels of BMP-7 may be responsible for cartilage protection and repair in OA. Measurements of plasma and/or synovial levels of BMP-7 could possibly serve as a biochemical parameter for determining disease severity and might be predictive of prognosis with respect to the progression of the osteoarthritic disease process. Additional studies may provide further information regarding the value of BMP-7 as a potential marker to monitor the course of OA.

It should be pointed out, however, that a limitation of this study is that the sample size was not large enough to make strong conclusions. Our preliminary data should be confirmed in a greater number of subjects. Additionally, incomplete assessment of potential confounders (age, gender, medical comorbidities) and the effect of joint sites other than the knee need to be taken into account. Another

Fig. 4 Plasma BMP-7 levels correlated with synovial fluid BMP-7 levels ($r=0.71$, $P<0.001$)

limiting factor of this study is that plasma and/or synovial levels of BMP-7 did not necessarily reflect the action of BMP signalling pathways. However, with the supporting evidence from other studies regarding the association between BMP-7 levels and the degree of systemic inflammatory response [2, 7, 18], it is likely that the elevated plasma and synovial BMP-7 levels found in primary knee OA patients may be involved in cartilage repair mechanisms and progression of cartilage degeneration. Finally, the female predominance of patients with OA may lead to a potential bias in comparing the levels of BMP-7 because controlled data obtained from a large sample size are currently not available.

In summary, patients with primary knee OA had elevated levels of plasma BMP-7 compared with healthy controls. We performed this study with the goal of relating plasma BMP-7 levels to the progression of knee OA. We showed that plasma BMP-7 concentrations significantly correlated with the magnitude of OA radiographic progression. BMP-7 measurement may not only serve as a biochemical marker of disease progression but also has the potential to contribute to the fundamental processes underlying the pathogenesis of primary knee OA. Further longitudinal investigations are warranted to elucidate the influence of BMP-7 on disease outcome.

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