Matrix metalloproteinase-3 (MMP-3) in synovial fluid with temporomandibular joint disorders

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Matrix metalloproteinase-3 (MMP-3, stromelysin) is known to degrade certain matrix components, including proteoglycans, fibronectin, laminin and collagens. We investigated the early signs of cartilage degradation by assaying MMP-3 in the synovial fluid of human temporomandibular joints with internal derangement (ID) or osteoarthritis (OA).

Synovial fluid was collected from the upper joint space by direct aspiration (non-diluted synovial fluid) or by pumping with lidocaine (diluted synovial fluid). MMP-3 was visualized by casein enzymography and measured by one-step sandwich enzyme immunoassay using an anti-human MMP-3 antibody that recognizes both active enzyme and inactive proenzyme. The concentration of MMP-3 with ID in both diluted and non-diluted synovial fluid was significantly greater than that with OA or in the asymptomatic controls (p<0.001). Enzymatically active components were detected in the non-diluted synovial fluid, but not in the diluted synovial fluid. Active MMP-3 was detected only in the non-diluted synovial fluid with OA, while inactive MMP-3 was observed in the non-diluted synovial fluid both with ID and with OA.

These results indicate that MMP-3 may be released as proenzyme into the synovial fluid during the early stages of degenerative changes in the TMJ. Subsequently, progressive TMJ destruction may induce the release of active MMP-3. We concluded that MMP-3 assay using synovial fluid may be a useful marker for the diagnosis of TMJ disorders. (J Osaka Dent Univ 2003; 37: 123–127.)

Key words: Synovial fluid; Stromelysin; Matrix metalloproteinase-3; Temporomandibular joint disorders

INTRODUCTION

The temporomandibular joint (TMJ) is a diarthrodial articulation between the condyle of the mandible and the articular eminence of the temporal bone. Synovial fluid lubricates the articular surfaces and nourishes chondrocytes. Synovial fluid analysis is a valuable diagnostic modality, as the information obtained reflects the physiology of the joint.¹⁻³ We have researched certain markers in human TMJ synovial fluid for diagnosis of the degree of TMJ disorders.⁴⁻⁵ Previously we examined the properties of hyaluronic acid in TMJ synovial fluid and reported that both the relative amount and the molecular weight of hyaluronic acid tended to decrease with TMJ osteoarthritis.⁴ We also demonstrated an increase in the amount of chondroitin sulfates released into the synovial fluid from articular cartilage with TMJ disorders.⁵ These results suggest the possibility of an increase in degradation of the extracellular matrix components in the dysfunctional TMJ.

Matrix metalloproteinases (MMPs) constitute a family of enzymes involved in the degradation of
the extracellular matrix in both the physiological and pathophysiological turnover of tissues. Proteinases are secreted by connective tissue cells as an inactive zymogen (proMMP), which is then activated by limited proteolysis. The proteolytic activities of MMPs are inhibited by their binding to tissue inhibitor of MMPs (TIMP). It is known that, in vivo, MMPs are present mainly in the inactive form, which is either the latent proform, or an inhibited complex with TIMP or α-2-macroglobulin. Among the MMPs, MMP-3 (stromelysin), which can degrade proteoglycans, gelatin and fibronectin, has been implicated as playing a pivotal role in joint-degrading diseases like arthritis.

To investigate the progressive signs of cartilage degradation, we quantitatively and qualitatively determined MMP-3 in the synovial fluid of human TMJ with internal derangement (ID) or osteoarthritis (OA).

MATERIALS AND METHODS

Sampling of the TMJ synovial fluid

The subjects for this study were selected from patients who visited Osaka Dental University Hospital, and who were diagnosed as having TMJ disorders by X-ray and magnetic resonance imaging (Fig. 1) (Table 1). Obtained consent was obtained from the patients based on the Helsinki Declaration.

Synovial fluid was collected from the upper joint space by direct aspiration (non-diluted synovial fluid) (Fig. 2) or by pumping with 1.5 mL of 2% lidocaine (diluted synovial fluid). After removal of the insoluble material by centrifugation at 3,000 rpm for 10 min, the synovial fluid samples were frozen at −20°C until used.

Enzymography

Proteolytic activities in synovial fluid samples were visualized by enzymography using a 10% SDS-polyacrylamide gel containing 0.1% casein by the method of Kubota et al. Samples were prepared for electrophoresis without heating or reduction. After electrophoresis, the gels were gently shaken in 2.5% Triton X-100 for 1 h at room temperature, and then incubated in assay buffer (100 mM Tris, 25 mM NaCl, 7 mM CaCl₂, pH 8.0) at 42°C for 96 h. Enzyme activity was detected as unstained bands in the Coomassie Brilliant Blue (CBB)-stained gel.

Measurement of MMP-3

MMP-3 in synovial fluid was measured by one-step sandwich enzyme immunoassay. The assay system used two simultaneous immunoreactions using a solid phase monoclonal antibody and a horseradish peroxidase-labeled monoclonal antibody (Fuji Chemical Inc., Toyama, Japan). The synovial fluid sample was incubated separately with a polystyrene ball coated with monoclonal antibody against human proMMP-3. The ball was removed, washed, and then incubated with tetramethyl-

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Age (mean)</th>
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</thead>
<tbody>
<tr>
<td>N</td>
<td>Normal</td>
<td>17-25 (21)</td>
</tr>
<tr>
<td>ID</td>
<td>Internal derangement with closed lock</td>
<td>16-51 (32)</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis with closed lock</td>
<td>20-60 (47)</td>
</tr>
</tbody>
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(yrs)

Fig. 1 Magnetic resonance imaging of the TMJ. T2-weight image shows the effusion has a high signal intensity.

Fig. 2 Non-diluted synovial fluid being collected by the direct aspiration method.
benzidine. The reaction was stopped by adding sulfuric acid, and absorbance was measured at 450 nm. The amount of MMP-3 was calculated from a standard curve using human proMMP-3 (Fuji Chemical Inc.) as a standard, and was then standardized as the amount of protein per sample. Specificity of this monoclonal antibody is 100% for human proMMP-3, and has negligible cross-reactivity with other MMPs and extracellular matrix macromolecules. Reactivity of this assay is 42% for active MMP-3, 54% for MMP-3 with TIMP-1, and 33% for MMP-3 with TIMP-2.12

Protein concentration of synovial fluid was measured by the pyrogallol red-molybdate complex method using protein assay rapid kit (Wako Chemicals, Osaka, Japan) according to manufacturer’s instructions. Bovine serum albumin was used as a standard protein.

RESULTS

Both in the diluted and non-diluted synovial fluid, the concentration of MMP-3 in the ID group was significantly higher (p<0.001) than that in the OA group or the asymptomatic controls (N group) (Fig. 3). There was no significant difference in the values between the OA group and the N group.

To determine whether synovial fluid MMP-3 is activated in TMJ disorders, a casein enzymography was performed. Typical enzymograms are shown in Fig. 4. Enzymatically active components were detected in the non-diluted synovial fluid, but not in the diluted synovial fluid. A proteolytic band near 50 kDa corresponding to active MMP-3 was detected only in the non-diluted synovial fluid with OA. Two positive bands near 80 kDa were observed in the non-diluted synovial fluid both with ID and with OA.

DISCUSSION

In this study, the MMP-3 level in the TMJ synovial fluid with ID was significantly higher than that with OA or with asymptomatic controls (Fig. 3). Research has demonstrated a significant increase in the synovial fluid MMP-3 levels of the knee joint with inflammatory arthritides.3,13-16 Ishiguro et al.17 also demonstrated that MMP-3 was markedly increased in the knee joint synovial fluid with rheumatoid arthritis (RA) compared with OA. In addition, they showed that synovial fluid MMP-3 concentration was closely correlated with the level of the soluble form of Fas ligand, which belongs to the tumor necrosis factor (TNF) family and participates in

![Graph showing concentration of MMP-3](image)

Fig. 3 Concentration of MMP-3 in TMJ synovial fluid. MMP-3 was measured by one-step sandwich enzyme immunoassay described in the methods. Closed symbols are individual data, bars represent the group mean ± SD. Significance was determined by the Mann-Whitney U test. *p<0.001

![Enzymographic image](image)

Fig. 4 Casein enzymography of MMP-3 in TMJ synovial fluid. Lane S shows a standard sample of active MMP-3. Active MMP-3 was only visualized in the non-diluted synovial fluid with OA.
apoptosis, indicating that the shedding of Fas ligand may be regulated by MMP-3 in the joint of patients with RA.

Recently, using experimental dogs, Schmidt and coworkers explored the levels of MMP-3, TIMP-1 and some proteoglycans in several cases of OA and inflammatory arthropathies. They suggested that although MMP-3 mediated matrix destruction is not of major importance in OA, MMP-3 seems to be a sensitive marker for local inflammation in arthritis. They also found a significant rise of MMP-3 in the synovial fluid of joints with RA, strongly correlating with the synovial mRNA content of interleukin (IL) - 1. Inflammatory cytokines such as IL-1 and TNF-α produced by activated synoviocytes, mononuclear cells or by the articular cartilage itself, significantly up-regulate MMP gene expression. Immunohistochemical analysis has demonstrated that MMP-3 is localized specifically on the surface of severely hypertrophic synovial membrane in the human TMJ tissues with OA. Therefore, the significant increase in the level of MMP-3 in the TMJ synovial fluid with ID that we observed in this study may be due to an increased release of MMP-3 from the synovial membrane caused by inflammatory stimulation.

Osteoarthritis is the most common joint disease in humans. It is characterized by a gradual loss of extracellular matrix components in the articular cartilage, such as collagen and proteoglycans. Presently, however, emphasis has been focused on enzymes exerting a strong influence on cartilage degradation. A previous study using a rabbit knee joint cartilage with experimental OA indicated that in OA, MMP-3 is initially upregulated in the synovium. This may play a pivotal role in the pathogenesis of cartilage lesions. In contrast, chondrocyte-derived MMP-3 is upregulated in the later phases of OA, contributing further to progression of cartilage lesions. Brama et al. investigated MMP-3 activity in synovial fluid in common joint disorders in the horse using a fluorogenic enzyme activity assay, and showed that the levels of active stromelysin in OA joints were about four times greater than in normal joints. This is in agreement with what would be expected with pathological matrix degradation. Using enzymography, we also found that a proteolytic band corresponding to active MMP-3 was only in the non-diluted synovial fluid with OA, not with ID. In OA patient knees, immunodetection of MMP-3 is demonstrated in a proportion of chondrocytes in the superficial zone that has degenerative matrix changes. Therefore, we think that the active MMP-3 detected in the synovial fluid with OA in this study was released from the degenerative cartilage.

Taken together, the results presented here indicate that MMP-3 may be produced simultaneously by synovioblasts during internal derangement and secreted as proenzyme into the joint cavity without being trapped by the extracellular matrix components. In addition, the progress of TMJ destruction may be caused by the release active enzyme from the cartilage extracellular matrix. These findings suggest that the MMP-3 assay using diluted and non-diluted synovial fluid may be a useful marker for diagnosis of TMJ disorders.

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