Changes in Synovial Fluid N-Acetyl-β-Glucosaminidase Activity in the Human Temporomandibular Joint with Dysfunction

Aiko KAMADA, Atsushi FUJITA, Kenji KAKUDO*, Joji OKAZAKI**, Masayasu IDA and Tetsuya SAKAKI

Department of Biochemistry, *First Department of Oral and Maxillofacial Surgery, **First Department of Prosthodontics, Osaka Dental University, 5-31 Otemae 1-chome, Chuo-ku, Osaka 540, Japan

β-Glycosidases (N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase, β-glucuronidase) were assayed in temporomandibular joint (TMJ) synovial fluid obtained from 23 patients with closed lock TMJ internal derangement (ID), four with closed-lock TMJ osteoarthritis (OA), and 13 with normal controls (N). Synovial fluid was collected from the upper joint space after injecting 1.5 ml of 1% lidocaine three times. The specific activity of N-acetyl-β-glucosaminidase increased significantly both with ID (p<0.01) and with OA (p<0.001), along with increases in the activity of N-acetyl-β-galactosaminidase (p<0.05 with ID and p<0.01 with OA) and in β-glucuronidase (p<0.05 both with ID and OA). The N-acetyl-β-glucosaminidase activity with OA was also significantly higher (p<0.001) than with ID. These findings suggest that N-acetyl-β-glucosaminidase activity in the TMJ synovial fluid reflects the degree of TMJ dysfunction. (J Osaka Dent Univ 1993; 27: 107-111.)

Key words: N-Acetyl-β-glucosaminidase; Temporomandibular joint; Synovial fluid; Internal derangement; Osteoarthritis

INTRODUCTION

The temporomandibular joint (TMJ) is a diarthrodial articulation between the condyle of the mandible and the articular eminence of the temporal bone. The synovial fluid lubricates articular surfaces and nourishes chondrocytes. Synovial fluid analysis is a valuable diagnostic modality, as the information obtained reflects the physiology of the joint1-9. We have researched certain markers in human TMJ synovial examined the properties of hyaluronic acid (HA) in TMJ synovial fluid and reported that both the relative amount and the molecular weight of HA tended to decrease with TMJ osteoarthritis4. These results suggest the possibility of a reduction in synthesis and/or an increase in degradation of the synovial fluid HA in the dysfunctional TMJ.

There is little information on HA metabolism of TMJ synovial fluid. As a
pilot study, we assayed the activity of β-glycosidases (in particular, N-acetyl-β-glucosaminidase, as well as N-acetyl-β-galactosaminidase and β-glucuronidase), which are lysosomal enzymes associated with the degradation of HA in human TMJ synovial fluid.

MATERIALS AND METHODS

Experimental samples
The subjects for this study were selected from patients who visited Osaka Dental University Hospital during 1991–1992, and who were diagnosed as having TMJ dysfunction by X-ray and magnetic resonance imaging (Table 1).

Table 1 Experimental subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnosis</th>
<th>Number of subjects</th>
<th>Age (yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>Normal</td>
<td>13</td>
<td>17-54</td>
</tr>
<tr>
<td>ID</td>
<td>Internal derangement with closed-lock</td>
<td>23</td>
<td>17-59</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis with closed-lock</td>
<td>4</td>
<td>22-59</td>
</tr>
</tbody>
</table>

Sampling technique
One percent lidocaine (1.5 ml) solution was injected into the upper joint space and the syring pumped three times to mix the solution with the synovial fluid. The resulting mixture then aspirated and collected. 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 assayed.

Enzyme assay
The activity of β-glycosidases was assayed using p-nitrophenyl (PNP)-β-glycosides as substrate following the method of Borooah et al.5. One part (100μl) of synovial fluid sample was incubated with 600 μl of substrate solution (3.5 mM substrate, 50 mM citrate buffer, pH 4.5) at 37°C for 40 min. The reaction was stopped by addition of 1.2 ml of 0.4 M NaOH-glycine buffer (pH 10.5), and then the released p-nitrophenol was measured at 410 nm using a Hitachi spectrophotometer Model 200-20. PNP-N-acetyl-β-glucosaminide, PNP-N-acetyl-β-galactosaminidase and PNP-β-glucuronide (Nacalai Tesque, Kyoto) were used as substrates for N-acetyl-β-glucosaminidase, N-acetyl-β-galactosaminidase and β-glucuronidase, respectively.

The concentration of protein in the synovial fluid was determined by the method of Lowry et al.6. The enzyme activity that released 1 nmol of p-nitrophenol per min was defined as one unit, and the enzyme activity corresponding to 1 mg of protein was taken to be the specific activity.

RESULTS

β-Glycosidase activity in normal TMJ synovial fluid
All of β-glycosidase activities examined were low in normal TMJ synovial fluid.
Fig. 1  *N*-Acetyl-β-glucosaminidase activity in TMJ synovial fluid.
Each value represents the mean±SD. Significant differences compared with normal controls (N) are shown as **p<0.01 or ***p<0.001.

Fig. 2  *N*-Acetyl-β-galactosaminidase (A) and β-glucuronidase (B) activities in TMJ synovial fluid.
Each value represents the mean±SD. Significant differences compared with normal controls (N) are shown as *p<0.05 or **p<0.01.

Fig. 3  Age-related changes in *N*-acetyl-β-glucosaminidase activity in normal TMJ synovial fluid.
compared with patients (Figs. 1 and 2). Fig. 3 shows the distribution of N-acetyl-β-glucosaminidase activity in normal TMJ synovial fluid with age and sex. The N-acetyl-β-glucosaminidase activity in the normal subjects was not related to age or gender.

**Changes in β-glycosidase activity with TMJ dysfunction**
All β-glycosidase activities examined in this study tended to increase with the degree of TMJ dysfunction (Figs. 1 and 2). The N-acetyl-β-glucosaminidase activity was 2.5 fold greater (p<0.01) with ID than with the controls, and 10.5 fold greater (p<0.001) with OA (Fig. 1). The N-acetyl-β-galactosaminidase activity was 2.9 fold greater (p<0.05) with ID and 5.1 fold greater (p<0.01) with OA, while the β-glucuronidase activity was 2.8 fold greater (p<0.05) with ID and 4.1 fold greater (p<0.05) with OA (Fig. 2). In particular, the N-acetyl-β-glucosaminidase activity with OA was much greater than with the controls, and was significantly higher than with ID (p<0.001).

**DISCUSSION**
A normal TMJ contains only a small amount of synovial fluid, as only a thin film of fluid covers the surfaces of the cartilage and synovium within the joint space. The volume of synovial fluid increases in disease states, creating an effusion, which permits aspiration and analysis of the fluid. Because normal joints do not have a sufficient volume of synovial fluid to permit aspiration, the knowledge of synovial fluid comes mostly from pathological effusions. However, we succeeded in recovering a mixture containing TMJ synovial fluid after injection of 1% lidocaine solution into the upper joint space, allowing us to carry out a biochemical study.

Normal synovial fluid contains high concentrations of HA, a long-chain polysaccharide consisting of repeating units of glucuronic acid and N-acetylglucosamine, which gives synovial fluid a high viscosity and plays an important role in joint lubrication. We found a correlation between the increase in β-glycosidase activities and the degree of the TMJ dysfunction, indicating that the degradation of HA is accelerated with this disease. These findings are consistent with the decrease in the amount and molecular weight of HA with TMJ osteoarthritis. The reason for the increases in the enzyme activities is unclear, but might be due, for example, to an increased permeability of the synovial membrane to plasma proteins (N-acetyl-β-glucosaminidase activity in serum is significantly higher than in TMJ synovial fluid), degradation of synovial membrane cells, or other factors. It is known that in most cases at least two isoenzymes of N-acetyl-β-glucosaminidase are present in various tissues. N-Acetyl-β-glucosaminidase is currently the most widely used urinary enzyme assay for the assessment of renal disease and the detection of nephrotoxicity. We hope that an understanding of the changes in the isoenzymes of synovial fluid N-acetyl-β-glucosaminidase in the dysfunctional TMJ will reveal the causes of increased in the enzyme activity, and anticipate that knowledge of synovial fluid N-acetyl-β-glucosaminidase will
help in the diagnosis of TMJ dysfunction.

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REFERENCES