Original Article

Expression of proteoglycan mRNA in human temporomandibular joint disks in temporomandibular disorders

Tomoaki Shibuya, Koji Kino* and Teruo Amagasa

Dept. of Maxillofacial Surgery, Maxillofacial Reconstruction and Function, Division of Maxillofacial and Neck Reconstruction, Graduate School, Tokyo Medical and Dental University, PO BOX 113-8549 5-45 Yushima 1-chome Bunkyo-ku, Tokyo, Japan. TEL: 81-3-5803-5502, FAX: 81-3-5803-0198

*Temporomandibular Joint Clinic, Faculty of Dentistry, Tokyo Medical and Dental University, PO BOX 113-8549 5-45 Yushima 1-chome Bunkyo-ku, Tokyo, Japan. TEL: 81-3-5803-5713, FAX: 81-3-5803-5713

TMJ disks contain decorin, and are fibroblastic tissue. If the disk was subjected to excessive compression in addition to tension, the pattern of gene expression became chondrocytic and expression of aggrecan was induced. Calcification would be initiated in the part of the disk experiencing the increased compression.

Key words: Proteoglycan, Decorin, Aggrecan

Introduction

The temporomandibular joint (TMJ) provides articulation between the mandible and cranium, and is associated with jaw movement and growth. Collagens1-2 and proteoglycans (PGs)3-8 have been investigated as the main components of the TMJ disk. PGs are a group of complex and diverse macromolecules composed of a core protein and peripheral glycosaminoglycans (GAGs). They have important roles in TMJ function.

The molecular and cellular basis of the pathophysiology of temporomandibular disorders (TMD), especially internal derangement, has not been widely investigated and is still not clear. Details of the expression of PG mRNA are unknown and no detailed papers on the subject have been presented. The purpose of this study was to investigate the expression of PG mRNA in human TMJ disks from patients with TMD.
Materials and Methods

Tissue Preparation: TMJ disks were collected from 11 TMJs in nine patients (six women and three men; age range 22-64 yr) with symptoms of TMD. All the patients had long histories of TMJ pain and dysfunction (Table 1), and had undergone diskectomy (bilaterally in two patients) under general anesthesia after unsuccessful conservative therapy and arthroscopic surgery. The patients agreed to enroll after being fully informed of the study method and aims. Total RNA was isolated from the disks using ISOGEN (GC) based on the method of Chomczynski et al.\textsuperscript{9}, then reverse-transcribed to cDNA using the SUPER SCRIPT Preamplification System (GiboBRL).

RT-PCR: PCR was performed using primers for the proteoglycans, aggrecan and decorin, whose DNA sequences have been reported previously\textsuperscript{10,11} (Table 2). PCR was performed using a Mini Cycler\textsuperscript{TM} (FUNAKOSHI), according to the manufacturer’s instructions. Briefly, single-stranded cDNA for one PCR was prepared from 0.5 ug of total RNA using an oligo (dT) primer and 1.25 U of reverse transcriptase. The cDNA product (10 ul) was diluted to a total of 50 uL of PCR mixture (10 mM Tris-HCl, pH 8.3 , 50mM KCl, 2 mM MgCl\textsubscript{2}, 200 uM each dNTP, and 0.15 uM each primer) containing 1.25 U of AmpliTaq polymerase (TAKARA). Amplification was performed in a thermal cycler at 94°C (5 min), followed by 30 cycles of 94°C (30s), 50°C (30s), and 72°C (60s), then 72°C (10 min).

Analysis of PCR Products: After amplification, aliquots of the samples were run on a 4.5% agarose gel, and amplified bands were revealed after staining with ethidium bromide. We sequenced the PCR products of the human TMJ disks by a direct sequencing method which used the same primers\textsuperscript{12}, enabling us to

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Patient No.</th>
<th>Diagnosis</th>
<th>Affected side</th>
<th>Age</th>
<th>Gender</th>
<th>Duration before operation</th>
<th>The particle of the disc which isolate RNA</th>
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<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>TMD</td>
<td>left</td>
<td>22</td>
<td>female</td>
<td>3year10months</td>
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<tr>
<td>2</td>
<td>2</td>
<td>TMD</td>
<td>right</td>
<td>26</td>
<td>male</td>
<td>2year8months</td>
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<tr>
<td>3</td>
<td>3</td>
<td>TMD</td>
<td>right</td>
<td>29</td>
<td>female</td>
<td>8year8months</td>
<td>normal</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>TMD</td>
<td>left</td>
<td>30</td>
<td>female</td>
<td>9year8months</td>
<td>normal</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
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<td>31</td>
<td>female</td>
<td>10years</td>
<td>normal</td>
</tr>
<tr>
<td>6</td>
<td>6</td>
<td>TMD</td>
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<td>31</td>
<td>male</td>
<td>1year8months</td>
<td>normal</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>TMD</td>
<td>right</td>
<td>31</td>
<td>female</td>
<td>4year2months</td>
<td>normal</td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>TMD</td>
<td>left</td>
<td>31</td>
<td>female</td>
<td>4year2months</td>
<td>normal</td>
</tr>
<tr>
<td>9</td>
<td>9</td>
<td>TMD</td>
<td>left</td>
<td>34</td>
<td>male</td>
<td>1year1month</td>
<td>calcification</td>
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<td>TMD</td>
<td>left</td>
<td>38</td>
<td>female</td>
<td>5years</td>
<td>normal</td>
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<tr>
<td>11</td>
<td>11</td>
<td>TMD</td>
<td>right</td>
<td>64</td>
<td>female</td>
<td>1year1month</td>
<td>normal</td>
</tr>
</tbody>
</table>

Table 2. The primers which used by PCR methods

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Nucleotide Positions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggrecan\textsuperscript{11}</td>
<td>S 5'−GATCCTTTCTACTTGGCCTCCTAC-3' 2325-2348</td>
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<tr>
<td></td>
<td>A 5'−TCTCCACTGCTGGAAGTCACCA-3' 2657-2680</td>
</tr>
<tr>
<td>Decorin\textsuperscript{10}</td>
<td>S 5'−CGGAATCAAAGATGGAG-3' 392−409</td>
</tr>
<tr>
<td></td>
<td>A 5'−GGTGATTTGATCAGC-3' 733-750</td>
</tr>
</tbody>
</table>

S: Sense primer, A: Antisense primer
recognize which PGs were present.

Results: In these human TMJ disks, a single band of PG mRNA was recognized in the PCR products of each sample, indicating the expression of decorin mRNA (Figure 1). Therefore all the RNA samples were intact. In the calcified part of the disk which was evident macroscopically, a single band of aggrecan mRNA was also recognizable (Figure 2). However, in the normal part of the same sample, no single band was recognized. Therefore, disks showing evident calcification expressed aggrecan mRNA.

Discussion

Generally, PGs are separated into high-molecular-weight (PG-Ls) and low-molecular-weight types (PG-Sms)\(^5,6\). PG-Ls include aggrecan\(^13\), which is distributed in cartilage, arteries, vascular endothelium, tendons and knee menisci\(^14-19\), and appears to be mainly a product of chondroblasts\(^20\). PG-Sms include decorin, which is a major PG-Sm of connective tissues\(^15,18,24,25\). Aggrecan plays a major role in determining the resistance of tissues to compressive load, and is involved in strong water-binding and electrostatic interactions. In vitro, cells subjected to compression synthesize aggrecan, and removal of compression results in loss of aggrecan. A compressive load stimulates and maintains the synthesis of aggrecan in specialized regions of the tendon\(^21-23\). The predominant PG in the tensional region of the tendon is decorin, and tension is necessary for maintaining decorin synthesis. This PG is associated with the linearly arranged collagen fibrils that are typical of tendon tissues\(^26\). In the Achilles tendon, aggrecan mRNA is present in the fibrous cartilage but absent from the mid-tendon, which is characterized by molecules typical of fibrous tissues\(^27\). A tendon may have a calcified part, just as part of an articular hyaline cartilage may be calcified\(^28\).

In the present study, we investigated the expression of PG mRNA using the RT-PCR method. Since RT-PCR requires two enzymatic steps and is very sensitive, the relative amount of original mRNA used for analysis needs to be considered carefully. In previous studies, decorin and aggrecan were recognized in the human TMJ disk by Western blotting\(^9\). We therefore suggested that there should be a good correlation between levels of expression of the genes and synthesis of their products in the human articular disk. In this study, expression of decorin mRNA was identified in the disk of patients with TMD. In the calcified particles of the disk, an aggrecan mRNA band was detectable visually. Even in the TMD of patients with a long history of TMJ pain and dysfunction, the pattern of gene expression in the disk was fibroblastic and included decorin. It resembled the mid-tendon part of the Achilles tendon, rather than fibrous cartilage. However, if the disk had been subjected to overload in addition to tension, the expression profile might have been different, perhaps being more chondrocytic and including aggrecan. With aging, cells similar to chondrocytes\(^29\) appear. However, our patients were not elderly, and so chondrification might extend into part of the disk and calcification might have been initiated under the influence of increased compression. Therefore, the level of expression of PGs mRNAs in the TMJ disk may be regulated by the mechanical environment in each part of the disk.

Even if other factors have to be considered, it may be concluded from the present results that the human TMJ
disk of TMD patients contains decorin, whose mRNA is recognized. If the disk is subjected to excessive compression in addition to tension, and tissue calcification is initiated, the synthesis of aggrecan mRNA increases in the disk. Immunohistochemistry or in situ hybridization would be useful for examining the presence of aggrecan in histologic specimens, and for determining whether tissue calcification has begun in any part of the disk subjected to increased compression, TMJ disks contain decorin, and fibroblastic tissue. If the disk is subjected to excessive compression in addition to tension, the pattern of gene expression will become chondrocytic, and expression of aggrecan will be induced. Calcification would be initiated in the part of the disk subjected to most compression.

References