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Changes in the expression of MMP-3, MMP-9, TIMP-1 and aggrecan in the condylar cartilage of rats induced by experimentally created disordered occlusion

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ABSTRACT

Objective: To investigate the effects of experimentally created disordered occlusion on the mandibular condylar cartilage in terms of histological morphology and expression of MMP-3, MMP-9, TIMP-1 and aggrecan.

Materials and methods: Eighty 8-week-old Sprague–Dawley rats were randomly divided into two experimental (Exp) and two control (Con) groups, with equal sex and number distribution as subgroups. In the Exp group, the disordered occlusion was created by orthodontically moving the first and third molars 0.8 mm away. Hematoxylin–eosin and immunohistochemical staining were performed on the mandibular condyles at the end of the 8th or 12th week. Gene expression was analysed by real-time PCR.

Results: Osteoarthritis-like lesions, typically seen as a cell-free area, were detected in the Exp group, predominantly in females. In the cell-free area, the immunopositive expression of MMP-3, MMP-9, TIMP-1 and aggrecan were absent. Hyper-proliferation changes, typically seen as conjunctive invaginations of chondrocytes, were also observed where immunopositive expression of the tested materials was strong. There were sex and time point related differences in gene expression. In the 8-week subgroup, the expression of MMP-3 decreased, while aggrecan increased in males; however, both MMP-9 and TIMP increased in the female group ($P < 0.05$). In the 12-week subgroup, the expression of MMP-3 increased, while TIMP, MMP-9 (male only) and aggrecan (female only) decreased ($P < 0.05$).

Conclusions: The present results indicate that the experimentally created disordered occlusion led to osteoarthritis-like lesions accompanied by changes in the expression of MMP-3, MMP-9, TIMP-1 and aggrecan in mandibular condyle cartilage with gender differences.

1. Introduction

Temporomandibular disorders (TMD) are frequently seen in dental practice. One severe pathological change seen in TMDs is the destruction of joint cartilage, known as osteoarthritis (OA), which is characterised by the degradation and loss of articular cartilage, hypertrophic bone changes with osteophyte formation and subchondral bone

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remodelling, and, at the clinical stage, chronic inflammation of the synovial membrane.[1,2] Changes in the condylar cartilage have been reported using different animal models.[3–6] Recently, a rat model with significant OA-like lesions in the mandibular condylar cartilage induced by the experimentally created disordered occlusion was reported.[7]

Cartilage metabolism is characterised by a highly regulated balance between the synthesis and degradation of various components in the extracellular matrix (ECM). Matrix metalloproteases (MMPs) are considered to be key enzymes in the degradation of the extracellular matrix (ECM)[8,9] and are capable of degrading the major components of the extracellular matrix at physiological pH. MMP-3 (stromelysin), which is secreted by fibroblasts, synovial cells and chondrocytes,[10,11] acts as the most important protease in cartilage matrix degradation.[12] MMP-3 not only degrades most components of the extracellular matrix, such as aggrecan, basement membrane, elastin, laminin, fibronection, etc., but also activates other MMPs in cartilage, such as MMP-9 (gelatinase B),[13] which uses type IV and V collagens as substrates and plays an important role in the development of OA.[14] Tissue inhibitors of matrix metalloproteinases (TIMPs), the natural endogenous inhibitors of MMPs, named TIMP-1, TIMP-2, TIMP-3, and TIMP-4, can bind MMPs with a 1:1 stoichiometry.[15] The imbalance between MMPs and TIMPs has been considered as a major factor in the development of progressive joint destruction.[16,17] Kanyama et al.[18] examined the level of MMPs and TIMPs in synovial fluid aspirated from patients with TMJ osteoarthritis and found a strong association between joints displaying OA and the presence of biologically active forms of MMP-1, MMP-3, and MMP-9. Yoshida et al.[19] demonstrated that the expression of MMPs in the synovial fluids from patients with symptomatic TMD was significantly higher than those in the normal control group. However, Kubota et al.[20,21] reported an increase in MMP-3 and MMP-9 in only a few synovial fluid samples taken from osteoarthritis TMJs.

Aggrecan, a large aggregating proteoglycan (PG) that binds to hyaluronan leading to a super molecular complex, is one of the major structural components of cartilage.[22] Struglics et al.[23] reported fragments of aggrecan in the synovial fluid from patients with osteoarthritis of the knee. Unilateral bite[6] and extraction of molars[3,4] increased the immunoreactivity of aggrecan in the rat condylar cartilage, but compressive forces, induced by a specially designed appliance, reduced the expression of aggrecan in the rat TMJ.[24]

In summary, MMP-3, MMP-9, TIMP-1 and aggrecan are expressed in joint cartilage, and their expressions are sensitive to the degenerative changes of the joint, such as in osteoarthritis. However, expression changes of these materials in OA remain inconsistent. In the present study, the rat model with experimentally created disordered occlusion, which was reported to be able to induce an OA-like lesion in mandibular condylar cartilage,[7] was adopted. The purpose was to investigate the possible changes in the expression of MMP-3, MMP-9, TIMP-1 and aggrecan in the condylar cartilage with induced OA-like lesions.

2. Materials and methods

2.1. *Animals and the establishment of the experimentally created disordered occlusion*

Animals were cared for according to the guidelines set by the Laboratory Animal Research Center of the Fourth Military Medical University. Eighty 8-week-old Sprague–Dawley rats (40 males weighing 200 ± 5 g and 40 females weighing 190 ± 5 g) were provided by the animal centre of the Fourth Military Medical University. According to the indications of Shen et al.,[25] the SD rats aged 8–20 weeks fell in the period of young adult human beings.

Eighty rats were divided randomly into two experimental groups (Exp) and two sham-operated control groups (Con), each containing ten males and ten females. In the experimental group, the first and third molars of the left maxilla and right mandible were moved mesially and distally, respectively, as previously reported.[7] Briefly, an elastic rubber band (Unitek™ Elastics, 3M Unitek, 1/8 #) was inserted between the first and second molars of the left side of the maxillary dentition and of the right side of the mandibular dentition. The rubber bands were carefully laid to be lower than the occlusal surface of the molars so that the occlusal contact relationship would not be interfered with. The first molars were gradually moved mesially, and the distance was about 0.8 mm between the first and the second molars 1 week later. The elastic rubber bands were then replaced with self-curing resin (Zhangjiang Biomaterial Co., Shanghai, China) to maintain the gaps between the first and second molars. Care was also taken to ensure that the resin was low enough not to interrupt the occlusion relationship. At the beginning of the 5th week, the same method was used to push the left maxillary and right mandibular third molars distally. In this way, two first and two third molars were moved away from their original places and no longer intercuspated their opposite molars (Fig. 1). In all of the operations, the mouth opening length was limited to within 15 mm. Each operation took less than 5 min.

All sham-operated control rats were subjected to all of the above procedures, except for those keeping the elastic rubber bands or self-curing resin between the molars.

Experimental animals, together with their age-matched controls, were sacrificed under deep anaesthesia by cardiac perfusion with isotonic sodium chloride and 40 g/L paraformaldehyde (pH 7.4) at the end of the 8th or 12th weeks after the start of the experiment, named accordingly as the 8- and 12-week subgroups. No rats showed any sign of disability, and all of them received the same standardised diet throughout the experimental procedure.

2.2. *Tissue preparation*

For histological morphology and immunohistochemistry examinations, the TMJs from four of the ten rats in each group were removed, post-fixed in 40 g/L paraformaldehyde for 24 h, decalcified in Krinstense' fluid (sodium formate 52.2 g, formic acid 174.2 mL, distilled water 1000 mL) for 1 week, dehydrated in graded alcohols, and embedded in paraffin. Serial sections of 5 μm in thicknesses were cut parallel to the sagittal plane of the mandibular condyle with a rotary