Effects of joint loading on extracellular matrix mRNA expressions in the mandibular condylar cartilage of growing rats

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【Introduction】

Mandibular condylar cartilage (MCC), an important target tissue in orthodontic treatment, is one of the growth sites showing the most prosperous growth in the cranium. In orthodontic clinic, by modifying the biomechanical environment of the temporomandibular joint directly or indirectly using an orthopedic device or a functional corrective device, control of the growth of the mandible which greatly influences maxillofacial morphology and occlusion formation is controlled doing. The mandibular condyle consists of fibrocartilage and contains three distinct cell populations: fibroblasts, the proliferative cells and chondrocytes, and shows a complicated reaction to changes in the biomechanical environment. We believe understanding the cellular changes due to altered joint loading of the MCC may contribute to our understanding of the mechanism underlying condylar growth modification in response orthodontic forces.

In this study, in order to clarify the morphological change of the MCC due to the joint load and the reactivity of the extracellular matrix, we used a growth model rat incisor occlusal elevation model, and histologically generated in the MCC by joint load and changes in mRNA expression of extracellular matrix were investigated.

【Material and Methods】

1, Joint loading on the TMJ

One hundred and four 7-week-old male Wistar rats were assigned randomly to control and bite plane groups. In order to increase the joint load to the temporomandibular joint, a resin occlusal plate was attached to the maxillary incisor part, and an occlusion modification model by occlusion elevation was prepared. Rats from each group were sacrificed after 1, 2, 3, and 4 weeks of wearing the appliance.

2, Tissue Preparation and Morphometry（n=3）

The TMJs of rats per time point in each group were dissected en bloc, in accordance with the Method, Paraffin-embedded specimens were cut sagittally in serial sections at a thickness of 7 μm and stained with toluidine blue (pH 4.1), and histologically examined. In addition, the thickness and cell number of each cell layer at the upper posterior part of the MCC was measured.

3, Quantification of DNA, GAG and collagen content（n=5）

MCC were collected from 5 rats. Samples placed in a papain digest solution at 60oC for 24 hours. The resulting digest material was analyzed for DNA using Hoechst 33258 and for GAG using a dimethylmethylene blue (DMB) assay. And analyzed for collagen content using a hydroxyproline assay as well.

4, Quantification of proteoglycan and collagen mRNA expressions（n=5）

DNA-free total RNA was extracted from the TMJ discs of 5 rats per time point in each group using the RNeasy Mini Kit (Qiagen, Hilden, Germany) and converted into cDNA using the Omniscript RT Kit (Qiagen). mRNA expression levels for proteoglycans and collagens at each growth stage were examined by real-time RT-PCR with the TaqMan probe.

5, Statistical Analysis

Statistical analysis was performed using SPSS 23 software. After confirmation of a normal distribution and quality of variance (F-test), the data from the 2 groups were analyze dusing Student's t tests.

【Results】

1, Changes in MCC Thickness and

Histological examination of 2 weeks bite plane groups, the proliferative cell layer was the Cell density decreased. The extracellular matrix showing metchromatic staining was reduced in the hypertrophic area.

In thickness measurement of MCC in bite plane group, the thickness increased in the fibrous layer and the proliferating cell layer, and the thickness decreased in the chondrocyte layer. In addition, the number of cells increased in one week of the proliferating cell layer.

2, Changes in DNA and GAG Contents

The DNA content increased significantly in the bite plane group after 1, 4 weeks. The GAG content did not differ significantly between the 2 groups throughout the experimental period.

3, Changes in collagen Contents

The collagen content did not differ significantly between the 2 groups throughout the experimental period.

4, Quantification of proteoglycan mRNA expressions

While the aggrecan mRNA levels decreased after 2 weeks, and the type Ⅱ collagen mRNA level decreased after 1, 2, 3 weeks. Also, the type Ⅲ collagen mRNA levels increased after 1, 3 weeks.

The biglycan and decorin, PRELP, mimecan, type I collagen mRNA levels increased after 1 weeks, and the fibromodulin, lumican, chondroadherin mRNA level increased after 1, 2 weeks.

【Discussion】

In this study, an incisal bite plane was used to alter the mechanical environment of the MCC. Wearing the bite plane increased the frequency and duration of the power stroke of incision, while eliminating the masticatory stroke. As a result, the bite

plane may have altered the joint loading on the MCC. The change in the thickness of the MCC was considered to be an increase in the number of cells constituting each cell layer or a change in extracellular matrix. In the previous study, fibroblasts in the fibrous layer and undifferentiated mesenchymal cells in the proliferating cell layer show proliferative activity in the MCC. In this study, although there was a clear tissue change in the proliferating cell layer and chondrocyte layer at 2 weeks, there was no change in the cell number, so that the change in the thickness of these cell layers was caused by the cell size and the extracellular matrix Capacity is considered to be involved. Expression of Collagen and proteoglycan mRNA increased type I and type III collagen as a response to mechanical environmental change of the MCC. Several histological studies in the past point out the relationship between mechanical stimulus and composition and composition of extracellular matrix. Therefore, the collagen distribution of the MCC may reflect the biomechanical and functional requirements of the tissue. In this study, biglycan, decorin, fibromodulin, lumican and mimecan, which showed increased mRNA expression, have been shown to be involved in the formation of collagen fibrils. These SLRPs are thought to be involved in the formation and remodeling process of fibrils of Type I and Type III collagen of the MCC to the change of the dynamic environment and contributed greatly to the change in the thickness of the proliferating cell layer. On the other hand, type II collagen mRNA expression decreased. Type II collagen and aggrecan are cartilage-specific extracellular matrix. Changes in the chondrocyte layer in this study showed that the differentiation of chondrocytes was suppressed by loading and the cells were not enlarged morphologically, so the size of the chondrocyte layer was reduced as compared with the control group. In addition, the decrease in hypertrophic chondrocytes producing aggrecan and type II collagen, which are extracellular matrix of the chondrocyte layer, decreased the thickness of the chondrocyte layer, which greatly affected the load resistance of the MCC ,and it was considered to be. However, there was no significant difference between the proliferating cell layer and the chondrocyte layer at 4 weeks. It was thought that this was the result of the tissue remodeling corresponding to the loading load caused by adaptation of the temporomandibular joint including the MCC and the surrounding tissue.

【Conclusion】

The incisor bite raising varied the mechanical load exerted on the MCC of the rat in the growing phase. As a result, differentiation from proliferating cell layer to chondrocyte and hypertrophication of chondrocytes were suppressed. On the other hand, the tissue structure of the MCC was changed by maintaining cell proliferation in the

proliferating cell layer and endochondral ossification in the hypertrophic chondrocyte layer. These changes suggested that the adaptation of the temporomandibular joint and surrounding tissue returns to the original tissue structure.