Original Article

Functional Lateral Shift of the Mandible Effects on the Expression of ECM in Rat Temporomandibular Cartilage

Tanapan Wattanachai; Ikuo Yonemitsu; Sawa Kaneko; Kunimichi Soma

ABSTRACT

Objective: To test the hypothesis that the effects of mechanical stress from a functional lateral shift of the mandible have no effect on the expression of two main condylar cartilage extracellular matrix components, type II collagen and aggrecan, in rats from early puberty to young adulthood.

Materials and Methods: Functional lateral shift of the mandible was induced in experimental groups of 5-week-old male Wistar rats, using guiding appliances. The rats were sacrificed at 3, 7, 14, and 28 days post appliance attachment. The condyles were immunohistochemically evaluated for type II collagen and aggrecan (the immunoreactive areas were quantified).

Results: As compared with the control group, on the contralateral condyles, the immunoreactivity of the experimental groups was significantly increased from 7 to 14 days. While on the ipsilateral condyles, the immunoreactive areas were significantly decreased throughout the experimental period.

Conclusion: A functional lateral shift of the mandible modulated the condylar cartilage extracellular matrix differently on each side of the condyle, which affected condylar morphology, growth, biomechanical properties, and even the susceptibility of the condylar cartilage to pathogenesis. (Angle Orthod. 2009;79:652–659.)

KEY WORDS: Condylar cartilage; Functional lateral shift of the mandible; Extracellular matrix

INTRODUCTION

A functional lateral shift of the mandible is the condition in which contacts of premature teeth cause the mandible to deviate laterally into maximum intercuspsation. It is frequently found associated with unilateral posterior crossbite in the deciduous and early mixed dentition of growing children. A unilateral crossbite was also reported in association with changes in condylar morphology in the temporomandibular joint (TMJ) in young adults, as well as with osteoarthrosis. Although the relationship between functional malocclusion and temporomandibular joint disorder (TMD) is still debatable, a high incidence of TMD signs and symptoms has been reported frequently in children and adolescents with a functional lateral shift of the mandible. Nonphysiologic mechanical loading and TMJ structural abnormalities are considered factors that contribute to TMD. These factors suggest that an underlying biological link between the loading from unilateral functional crossbite and TMD still is not understood.

The effects of a functional lateral shift of the mandible on growth, development, and remodeling of the TMJ condylar cartilage have greatly concerned researchers. Condylar cartilage is well known for its adaptability in remodeling in response to external stimuli during or after growth. Changes in condylar size, cartilage thickness, proliferation, and erosion also are affected by a functional lateral shift of the mandible. Because the condylar cartilage is sensitively responsive to load, these results indicated changes in the
stimuli. However, the effects under functional lateral shift of the mandible still are overlooked. The aim of this study was to investigate the effect of functional lateral shift of the mandible on the two main condylar cartilage extracellular matrix components—type II collagen and aggrecan expression—in rats from early puberty to young adulthood by means of immunohistochemistry.

MATERIALS AND METHODS

Experimental Model

The animal protocol was approved by the Animal Ethics Committee of Tokyo Medical and Dental University. Forty 5-week-old male Wistar rats were randomly divided into experimental (n = 20) and control (n = 20) groups. Each rat in the experimental group was fitted with a resin-plate guiding appliance attached to the upper incisors. This plate was designed to produce a lateral functional shift of the mandible 2 mm to the left upon jaw closure and a metal cap to prevent lower incisor attrition (Figure 1). Normal rats with no appliance served as the control. All rats were fed ground pellets and were given water ad libitum. The appliance in the experimental rat.

Figure 1. The appliance in the experimental rat.

Histologic Tissue Preparation

The animals were anesthetized and were intracardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). TMJs were dissected and immersed in the same fixative overnight, decalcified in a 4% ethylenediaminetetraacetic acid (EDTA) (pH 7.4) at 4°C for 6 weeks, and embedded in paraffin. Tissue blocks were cut frontally at a 6 μm thickness by a microtome (RM2155; LEICA Co. Ltd., Nussloch, Germany) and were mounted on silane-coated slides. Nine serial sections from the center of the anterior-posterior width of the condyle were selected. Then, each of the three sections was reacted with hematoxylin and eosin and antibody to type II collagen and aggrecan. Because there was no difference between ipsilateral and contralateral condyles on the nonappliance control, control samples were drawn only from the left-side condyle.

Immunohistochemistry

All deparaffinized sections were digested with testicular hyaluronidase (Sigma, Tokyo, Japan), at 25 mg/mL in PBS for 30 minutes at 37°C. For aggrecan immunostaining, the sections were treated further, as in the previous protocol. Then, the sections were incubated with antibodies against type II collagen (diluted at 1:500; LSL, Tokyo, Japan) and aggrecan (12/21/1-C-6 at 1:10; Developmental Studies Hybridoma Bank, Iowa City, Iowa, USA), overnight at 4°C. They were visualized through the biotin-streptavidin method (Histofine MAX-PO kit; Nichirei, Tokyo, Japan) and were stained with 3,3'-diaminobenzidine. For aggrecan staining, the sections were counterstained with hematoxylin.

Histomorphometry and Quantitative Analysis

The condyle was divided into three regions—lateral, central, and medial—by a diameter line and 60 degree angle lines from the center point (Figure 2). Images were captured with the cell layer parallel to a measurement frame of 250 × 350 μm (Nikon DXm1200; Nikon, Kanagawa, Japan) at the central region of the contralateral condyle and at the lateral region of the ipsilateral condyle, where most changes occurred, as described by Sato et al. The immunostained area was quantified by Image J 1.38 software (NIH, Bethesda, Maryland, USA). Data were analyzed by the Mann-Whitney U-test (P < .05) and the Statistical Package for the Social Sciences (SPSS), version 16.0 (SPSS Inc., Chicago, Ill, USA). The data were collected again 1 month later, by the same observer, to test the method of error (ME) with the formula, ME = \sqrt{\sum d^2/n}, where d is the difference between two registrations of a pair, and n is the number of double registrations. Paired t-tests were performed to compare the two registrations. No statistically significant differences were noted among the registrations.
RESULTS

Hematoxylin and Eosin Staining

Condyles on the experimental groups had morphologic changes at every experimental time point. The most remarkable changes were observed on day 14 post appliance attachment (Figure 3), as reported by Sato et al.12 As compared with the control group, the shape of the contralateral condyle became more prominent, especially at the central region. The ipsilateral condyle was flattened at the lateral region.

Type II Collagen

Type II collagen immunoreactivity appeared as a curved band covering the area of articular cartilage (Figure 4). It was clearly detected in the transitional and hypertrophic layers20 (Figure 5). In the control group, type II collagen expression decreased over the experimental period in both lateral and central regions (Figures 4b,e; 5a,c,e,g; and 6). In the experimental group, drastic changes occurred in the central region of the contralateral condyle (Figure 4a,d) and in the lateral region of the ipsilateral condyle (Figure 4c,f) when compared with the control group (Figure 4b,e). The immunostaining area in the central region of the contralateral condyle was significantly increased between 7 and 14 days post appliance attachment, with obvious enlargement of the chondrocyte in the hypertrophic layer before the stained area decreased to a slightly higher level than in the control; slight chondrocyte enlargement occurred at day 28 (Figures 5a–d, 6a). In the lateral region of the ipsilateral condyle, the immunostained area was decreased gradually between 3 and 28 days, with the chondrocyte in both transitional and hypertrophic layers gradually diminishing and almost vanishing by day 28 (Figures 5e–h, 6b), as compared with the control group.

Aggrecan

Immunostaining for aggrecan showed a similar distribution to that of type II collagen. As compared with the control group, a marked change in immunoreactivity in the experimental group occurred in the central region of the contralateral condyle and in the lateral region of the ipsilateral condyle (Figure 7). The expression of aggrecan in the central region of the contralateral condyle was increased significantly on days 7 and 14, while that in the lateral region of the ipsilateral condyle continued to decrease throughout the experimental period (Figures 8 and 9).

DISCUSSION

This model has been verified by various authors in terms of its effectiveness in inducing functional lateral shift of the mandible in rats.10–12 The 2 mm shift distance of the mandible has been proved to cause no nutritional problem.12 During the experimental period, no significant body weight differences were noted.
Figure 4. Type II collagen immunostaining. (a–c) At 14 days post appliance attachment. (d–f) At 28 days. The immunostained area in the central region (black arrowhead) of the experimental contralateral condyles increases on days 14 and 28 (a and d) compared with that of the control group (b and e). The immunostained area in the lateral region (white arrowhead) of the experimental ipsilateral condyles decreases on days 14 and 28 (c and f) compared with that of the control group (b and e). Bar = 300 μm; original magnification, 40×.

Figure 5. Type II collagen immunostaining at 100× magnification. (a–d) At the central region of the contralateral condyle. (e–h) At the lateral region of the ipsilateral condyle. (a, b, e, f) At 14 days. (c, d, g, h) At 28 days. (a, c, e, g) The control group. (b, d, f, h) The experimental group. T, transitional layer; H, hypertrophic layer. Bar = 100 μm.
among the groups (data not shown). The experimental period was designed to span the transition from early puberty (5 weeks old) to young adulthood (9 weeks old), including the age point at which the highest growth of the condyle occurs histologically in the frontal plane (7 weeks old). This experimental period enabled study of the effects of functional shift on ECMs from peak growth of the condyle to its retardation, when adulthood is entered.

It is well accepted that the condylar cartilage extracellular matrix and its parent cells, chondrocytes, respond to mechanical stimuli in various in vivo and in vitro experimental models. This study showed the effects of functional lateral shift of the mandible on the two main extracellular matrix components of the condylar cartilage. In the central region of the contralateral side, the area of extension force, the increase in expression of aggrecan could be correlated with increased synthesis of glycosaminoglycan in the posterior region of the condylar cartilage in response to mandibular advancement. In the lateral region of the ipsilateral condyle, the area of compressive force, the decrease in type II collagen and aggrecan expression can be correlated with reports of decreased expression under compressed retrusion of the condyle.

In terms of underlying mechanisms, type II collagen and aggrecan are metabolite products of mature chondrocytes. Progenitor cells in the perichondrium of the mandibular condyle proliferate and differentiate into chondroblasts, then mature and hypertrophy to become chondrocytes, which are responsible for the synthesis and integrity of these ECM molecules. After matrix mineralization, this part of the cartilage is replaced by bone via erosion. Thus, it is conceivable that rates of chondrocyte proliferation and erosion may affect the presence of these ECMs. Study of cell dynamics, with the same model used by Sato et al., also supported this view. These results revealed that on the central region of the contralateral side, the proliferation found increased at days 7 and 14, and erosion decreased throughout 28 days of the experiment. On the contrary, the lateral region of the ipsilateral side exhibited decreased proliferation at days 14 and 28 and increased erosion throughout the experimental period, as compared with controls. These results also imply that different types of force on each side of the condyle have governed the various remodeling responses.

In this study, increased expression of both ECMs in the central region of the contralateral condyle was found, in accordance with gradual enlargement of the chondrocytes from the beginning of the experiment to their maximum expression and chondrocyte size on day 14. These findings are consistent with a previous report of the association between increasing cartilage matrix production and chondrocyte enlargement. At day 28, the expression of both ECMs and chondrocyte size recovered to a level close to that in the control group. A possible reason for this phenomenon is adaptation, which occurred as adjustment and remodeling to normal as soon as a new functional equilibrium was achieved.

On the other hand, the continuous decrease in ECMs in the lateral region of the ipsilateral condyles is compatible with previous in vitro investigations, which showed that ECM synthesis decreased when high-magnitude intermittent compressive loading was applied. Therefore, it is logical that there must be excessive compressive loading in this area, leading to a dysfunctional type of remodeling rather than to physiologic remodeling of the condylar cartilage on this side. Moreover, it could be assumed that besides the directional difference of forces on the ipsilateral and contralateral, the magnitude difference seen in forces on both sides must be responsible for dissimilar expressions of ECMs on the two condyles.

ECM is essential in terms of volume growth of the condyle via the interstitial mode. It contributes elasticity and resilience to the condyle through the role of

Figure 6. Quantification of the immunoreactive area for type II collagen. (a) Central region of the contralateral condyle. (b) Lateral region of the ipsilateral condyle. ** P < .01; n = 5.
Figure 7. Aggrecan immunostaining. (a–c) At 14 days post appliance attachment. (d–f) At 28 days. The immunostained area in the central region (black arrowhead) of the experimental contralateral condyles increases on days 14 and 28 (a and d) compared with that of the control group (b and e). The immunostained area in the lateral region (white arrowhead) of the experimental ipsilateral condyles decreases on days 14 and 28 (c and f) compared with that of the control group (b and e). Bar = 300 μm; original magnification, 40×.

Figure 8. Aggrecan immunostaining at 100× magnification. (a–d) At the central region of the contralateral condyle. (e–h) At the lateral region of the ipsilateral condyle. (a, b, e, f) At 14 days. (c, d, g, h) At 28 days. (a, c, e, g) The control group. (b, d, f, h) The experimental group. T, transitional layer; H, hypertrophic layer. Bar = 100 μm.
type II collagen and aggrecan, to withstand multidirectional forces exerted on the condyle during function. Therefore, the existence of these major ECM components in cartilage tissue directly affects the shape and biological properties of the condyle.

Our results demonstrated remarkable morphologic changes in the condylar head on the ipsilateral side. In patients with mandibular displacement with TMD symptoms, condylar morphologic changes and disc displacement were also found to be higher on the mandibular deviated side. Pullinger et al suggested that adaptation may be made at the expense of the articular disc through the development of internal rearrangement, including a few cases that eventually progress to arthrosis. The decrease in type II collagen and aggrecan shown in this study is consistent with that seen in mechanically induced osteoarthrotic cartilage. Through integration of all evidence and consideration of the fact that the adaptive capacity of condylar cartilage decreases upon aging, it is possible that the condyle on the ipsilateral side is more susceptible to joint pathogenesis than a normal one. Therefore, functional crossbite correction is advisable, to protect condylar cartilage health.

CONCLUSIONS

• Functional lateral shift of the mandible affected remodeling of the condyle through changes in expression of type II collagen and aggrecan in the central region of the contralateral side and the lateral region of the ipsilateral side.
• The condyle on the contralateral side tended to exhibit adaptive remodeling to the new balance. When the ipsilateral condyle shows a tendency toward dysfunctional remodeling, caused by continuously decreasing type II collagen and aggrecan, it may become more vulnerable to pathogenesis.

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