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The influence of masseter activity on rat mandibular growth

Ikuo Yonemitsu *, *Takeshi Muramoto, Kunimichi Soma*

Orthodontic Science, Department of Orofacial Development and Function, Division of Oral Health Sciences, Graduate School, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan

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ABSTRACT

Many studies have shown that mandibular and condylar growth is affected by compressive forces on mandibular bone and the condyle. It has been reported that chondroblastic differentiation and proliferation in chondrocytes play important roles in condylar growth. However, the influence of reduced compressive force on chondroblastic proliferation and mandibular bone formation is not fully understood. Thirty-six 3-week-old male Wistar rats were used in this study. In the experimental group, the masseter muscles were bilaterally resected to evaluate the influence of masticatory force on mandibular and condylar bone morphology. Six weeks after the operation, while the rats were in the pubertal growth stage, lateral X-rays were taken to analyze the skeletal pattern of the mandible. The form of the condyle and the thickness of the chondroblastic layers were evaluated by toluidine blue staining. Chondroblastic proliferation was identified by insulin-like growth factor-1 receptor (IGF-1r) immunostaining and bone resorption of the condyle was assessed by measuring tartrate-resistant acid phosphatase (TRAP) activity.

Lateral X-rays of the mandible showed that rats in the experimental group tended to have large mandibular plane angles. The chondroblastic layer in the condyles of the experimental group rats was thinner than in the control group. The expression of IGF-1r immunopositive cells in the experimental group was significantly lower than in the control chondrocytes, and the number of TRAP-positive cells was significantly higher in the condylar bone of the experimental group. We conclude that masseter muscle activity is closely related to mandibular morphology during growth.

1. Introduction

Clinical studies have shown that the direction and amount of mandibular growth is regulated not only by genetic factors but also by environmental factors.[1–4] For example, myotonic dystrophy patients, who have lower activity in the masseter muscles, are characterized by a large mandibular plane angle and abnormal bone changes.[1–3] At the other end of the scale, increased function of the masticatory muscles is associated with an anterior growth rotation pattern of the mandible and well-developed angular coronoid and condylar processes.[4]

The influence of masticatory muscles on the mandibular condyle has been previously studied using various experimental animal models[5–9] and has been shown to cause various changes in the skeletal pattern of the mandible. Bilateral resection of the jaw-closing muscles caused shortening of the ramal height and elongation of the molars.[5] Bilateral resection of the masseter muscles in rats resulted in a skeletal pattern with an open bite and a decrease in chondrocytes.[6,7] Denervation of the masseteric nerve in rats stunted the bone formation of the gonial region, increased vertical facial height and caused elongation of the molars.[8] In growing rats fed a soft

* *Corresponding author*. Tel.: +81 3 5803 5963; fax: +81 3 5803 5963.
E-mail address: yoneorts@tmd.ac.jp (I. Yonemitsu).
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diet, a decreased growth rate was found in the gonial angle of the mandible.[9]

The condylar cartilage acts as a regional adaptive growth site during mandibular growth.[10] It differs from other cartilage such as nasal septal cartilage, epiphyseal growth plates and femoral head cartilage, which are defined as primary cartilage. Primary cartilage appears earlier in embryonic development and is thought to have a relatively independent growth potential and to be less sensitive to functional factors. Condylar cartilage is defined as secondary cartilage. Secondary cartilage growth is more susceptible to applied loads than primary cartilage growth.[11] Mandibular cartilage is considered to be highly adaptive to biomechanical forces.

In vitro, it has been reported that intermittent compressive loading on condyles may induce type I collagen and fibronectin production by chondrocytes.[12] Moreover, compressive forces caused changes in the proliferation of chondrocytes and matrix synthesis in the mandibular condylar cartilage of rats.[13,14]

Cartilage growth is also regulated by various growth factors and regulatory factors expressed in chondrocytes.[15–23] Insulin-like growth factor-1 (IGF-1) is one of the growth factors that regulates the proliferation and differentiation of chondrocytes. While IGF-1 is mainly synthesized in the liver, it is also produced locally in the proliferative cells of the epiphyseal growth plate.[15,16] The chondrogenic potency of IGF-1 is evident from previous experimental studies which found that administration of IGF-1 to young rats was followed by accelerated chondrogenesis in the condylar cartilage and the long bone epiphyseal cartilage.[17–20] IGF-1 also plays an important role in preventing chondrocyte apoptosis. Disruption of the IGF-1 receptor is associated with severe growth retardation and delayed bone development.[24,25]

The aim of this study was to investigate the effects of loading of masticatory muscle forces that produce changes in the pattern of vertical facial morphology and changes in the condyle, using an animal experimental model. In addition, we used a bilateral masseter muscle resection model in order to weaken masticatory muscle forces and detect changes in IGF-1r expression in chondrocytes and tartrate-resistant acid phosphatase (TRAP) activity in the condylar bone, to reveal one of the mechanisms of condylar morphological change during the pubertal growth stage in vivo.

2. Materials and methods

2.1. *Animal and tissue preparation*

Animal protocols were approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University, and the experiment was carried out under the control of the University's Guidelines for Animal Experimentation.

Thirty-six 3-week-old male Wistar rats were used for this study. They were randomly divided into two groups. In the experimental group ($n = 20$), the masseter muscles were bilaterally resected, using the same model reported previously by Monje et al.[7] Before surgery, all animals were deeply anaesthetized with diethyl ether and intraperitoneal injection of 8% chloral hydrate using 1 ml/200 g of body weight. After shaving the area, they were dissected to visualize the masticatory muscles. All superficial and deep portions of the masseter muscles were bilaterally cut off at the end and removed without damaging any major blood vessels or nerves around the muscles. Then, opened wounds were sutured. At the conclusion of the operation, amoxicillin (ICN Biomedicals Inc., Ohio, USA) (9 mg/60 g of body weight) was injected to prevent infection. The second group of rats ($n = 16$) served as a control.

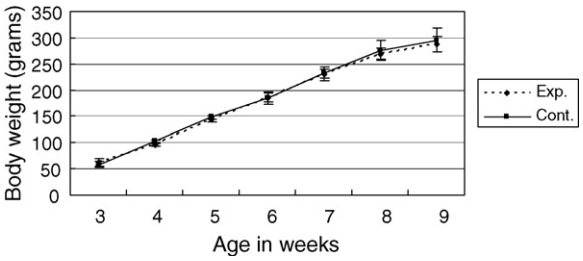

Fig. 1 – Changes in body weight of rats during the experimental period. Values are mean ± S.D. There were no significant differences between the two groups at any stage.

Effects on morphological changes were evaluated 6 weeks after the operation, while the rats were in the pubertal growth stage. The rats were fed pellets only, and body weight increased with no significant difference between the groups during this study (Fig. 1). The rats of the experimental group ate pellets with the same masticatory pattern in the jaw movement.

At the end of the experimental period, all the animals were deeply anaesthetized and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Heads of the rats were immersed in the same fixative overnight. The fixed specimens were sectioned mid-sagittally and subjected to soft X-ray radiography (40 cm, 25 kV, 2 mA, 45 s) in a SOFTEX CMB-2 (SOFTEX Co. Ltd., Tokyo, Japan) (Fig. 2). They were then decalcified in 4.13% EDTA at 4 °C for 6 weeks. Some condylar areas were cut, embedded in paraffin and cut sagittally into 6 μm-thick sections. The other condyle specimens were soaked in 30% sucrose solution in 0.01 M phosphate buffer saline (PBS; pH 7.4) at 4 °C overnight, cut sagittally into 40 μm-thick sections with a freezing microtome (Yamato Kohki, Saitama, Japan), collected in PBS and treated as free-floating sections.

2.2. *Radiological analysis of the mandible*

In the lateral X-ray of the mandible, the following four lengths and the angle of the mandible were measured with NIH image software (Fig. 3).[6,7,26]

L1—ramal height: from point Cd (the superior point of condyle) to line M (mandibular plane).
L2—condylar head length: from the posterosuperior to the anterosuperior border of the condyle, which is vertical to line C (between the deepest anteangular notch and Cd).