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The imbalance of masticatory muscle activity affects the asymmetric growth of condylar cartilage and subchondral bone in rats

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ABSTRACT

Objective: To examine the effects of imbalance of masticatory muscle activity of the rat mandible on the condylar cartilage and subchondral bone during the growth period.
Design: Forty 5-week-old male Wistar rats were randomly divided into experimental ($n = 20$) and control ($n = 20$) groups. In the experimental group, the left masseter muscles were resected. The rats were sacrificed at 7 or 9 weeks of age in both groups. Microcomputed tomography was used to determine the three-dimensional morphology and cancellous bone structure. For histological and histochemical examination, 5-μm-thick serial frontal sections of the condyle were stained with toluidine blue and immunostained with asporin and TGF-β1 to evaluate the promotion and inhibition of chondrogenesis.
Results: In the experimental group, microcomputed tomography analysis showed asymmetric growth; the resected side condyles showed degenerative changes. Histological analysis showed that the total cartilage in the central region of the resected side was significantly thinner than in the non-resected side in the experimental group, as well as in the control group. Compared with the control group, the expression of asporin was significantly higher in the resected side, and significantly lower in the non-resected side. In contrast, the expression of TGF-β1-immunopositive cells in the non-resected side was significantly higher than in the resected side and the control group.
Conclusions: These findings imply that lateral imbalance of masseter muscle activity lead to inhibition of chondrogenesis and induce asymmetric formation of the condyle during the growth period.

1. Introduction

The temporomandibular joint (TMJ) is a bilateral synovial articulation, the most common and most movable type of joint in the body, and is subjected to a variety of loads during jaw movements (Beek, Koolstra, van Ruijven, & van Eijden, 2001). Functionally, the TMJ performs not only hinge movements but also sliding movements; therefore, it is considered that compressive, shearing, and other complex forces are exerted on the mandibular condyle during masticatory function.

Condylar cartilage acts as a part of the articulatory joint and as a major growth site for the mandible. Growth and development of the mandibular condyle substantially influence morphogenesis of the craniofacial skeleton and function of the TMJ (Hinton, 1991). Designated as secondary cartilage, condylar cartilage differs from other cartilaginous tissues in its histological organization, its response to biomechanical stress and humoral factors, and its mechanisms of proliferation, differentiation and calcification (Berraquero, Palacios, Gamallo, de la Rosa, & Rodriguez, 1995).

Articular cartilage requires motion and joint loading to maintain its proper physical and biochemical properties (Leroux et al., 2001). In response to injury or extraordinary mechanical stress, cartilage cells decrease in number (Griffin & Guilak, 2005). Because articular cartilage lacks nerves and blood vessels, as well as lacking in self-healing ability, growth factors allow chondrocytes to differentiate to compensate for this decrease.

The transforming growth factor-β (TGF-β) family consists of over 35 factors, including TGF-β, activins and bone morphogenetic proteins (de Caestecker, 2004). These factors play vital roles in the development and homeostasis of various tissues; regulation of cell proliferation, differentiation, apoptosis and migration; and control of extracellular matrix synthesis and degradation (Blaney Davidson, van der Kraan, & van den Berg, 2007; Redini et al., 1991). Moreover, these factors mediate cell and tissue responses to injury and modulate immune functions (Javelaud & Mauviel, 2004).

Abbreviations: Micro-CT, microcomputed tomography; OA, osteoarthritis; PBS, phosphate buffered saline; PLAP-1, periodontal ligament-associated protein-1; SLRPs, small leucine-rich proteoglycans; TGF-β, transforming growth factor-β; TMJ, temporomandibular joint.

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However, the unlimited action of the growth factor causes new problems such as abnormal cartilaginous increase, ossification, and tumor formation, so that it is necessary to regulate the system of chondrogenesis (Kizawa et al., 2005). Asporin is expressed in various tissues, including articular cartilage, aorta, uterus, heart and liver, with especially high expression in cartilage (Lorenzo et al., 2001; Henry et al., 2001). Also known as periodontal ligament-associated protein-1 (PLAP-1), asporin has been investigated as a regulator of TGF-β1, and is a new member of the family of small leucine-rich proteoglycans (SLRPs) (Lorenzo et al., 2001; Yamada et al., 2007; Iozzo & Murdoch, 1996). Asporin has been reported to be involved in the development and progression of knee osteoarthritis (OA) by suppressing the anabolic action of TGF-β1 (Kizawa et al., 2005; Lorenzo et al., 2001; Jiang et al., 2006), but few studies have revealed any relationship between asporin and TMJ OA.

Many studies have been conducted investigating the association between facial morphology and muscle function (Odman, Mavropoulos, & Kiliaridis, 2008; Kim et al., 2008; Navarro, Delgado, & Monje, 1995; Ingervall & Thilander, 1974). Optimal masticatory muscle force during growth is necessary for normal mandibular growth (Monje, Delgado, Navarro, Miralles, & Alonso del Hoyo, 1994), and masticatory muscle function is a determinant of bone quality in the growing mandible (Bresin, Kiliaridis, & Strid, 1999; Poikela, Kantomaa, Tuominen, & Pirttiniemi, 1995).

Mandibular asymmetry has been related to the experimental unilateral removal of the masseter muscle in animal models (Rodrigues, Traina, Nakamai, & Luz, 2009; Avis, 1961). Similar deformities or asymmetries have been produced by resecting either the muscles (Sarnat, 1988) or the nerves of mastication (Kitagawa et al., 2002).

According to previous clinical studies, patients with facial asymmetry due to severe unilateral hypoplasia of the masticatory muscles show a tendency toward chewing on the normal side. The radiographic findings of a shortened ramus, smaller condyle and underdeveloped gonion on the hypoplastic side were consistent with previous studies (Sakamoto & Yoda, 2004; Parmar, Watkinson, & Fieldhouse, 1996).

Although it is known that changes in mechanical stress affect cartilage metabolism, the mechanism is still unclear. There is little evidence demonstrating the presence and immunolocalization of asporin within the area of the TMJ. Therefore, the aim of this study was to examine the effects of masticatory muscle imbalance on the morphogenesis of the condyle and localization of asporin and TGF-β1 in the condylar cartilage.

2. Materials and methods

2.1. Animals and experimental design

The experimental protocol involving animals was approved by the Institutional Animal Care and Use Committee of Tokyo Medical and Dental University (#015177A), and the University's Guidelines for Animal Experimentation were followed throughout the study. Forty 5-week-old male Wistar rats were randomly divided into two groups. In the experimental group ($n = 20$), the left masseter muscles were resected, using a method reported previously (Yonemitsu, Muramoto, & Soma, 2007). The rats were fed pellets only. Briefly, the rats were deeply anesthetized with diethyl ether and an intraperitoneal injection of 8% chloral hydrate using 1 ml/200 g of body weight. After shaving a wide area in the left gonial region, an incision was made and the masticatory muscles were exposed. All superficial and deep portions of the left masseter muscles were excised at each end and removed without damaging any major blood vessels or nerves around the muscles. The wounds were sutured, and amoxicillin (ICN Biomedicals Inc., Ohio, USA) (9 mg/60 g of body weight) was injected to prevent infection. The other group of rats ($n = 20$) served as control.

Morphological changes were evaluated 2 and 4 weeks after surgery (at age 7 and 9 weeks), while the rats were in the pubertal growth stage.

2.2. Microcomputed tomography analysis

The animals were perfused intracardially with 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.4). To detect the three-dimensional morphology and cancellous bone structure of the condyles, we used microcomputed tomography (micro-CT) (SMX-100CT, Shimadzu, Kyoto, Japan) with an output of 75 kV and 140 mA. Frontal cross-sections were taken initially followed by three-dimensional reconstruction to provide a histomorphometric view. The region of interest for the micro-CT analysis was determined as the midpoint of the maximum width between the lateral and medial poles of the condyle. A software package (TRI/3D-BON, Ratoc, Tokyo, Japan) was used to analyze the results (Kuroda et al., 2011). Three cubic frames (each 0.3 mm × 0.3 mm × 0.3 mm) under the osteochondral interface were located at the middle of the lateral, central and medial aspects of the mandibular condyle. Within the selected frames, we calculated bone volume/trabecular volume (BV/TV), bone mineral density (BMD), bone surface/bone volume (BS/BV), Tb.Th (trabecular thickness), trabecular number (Tb.N) and trabecular separation (Tb. Sp).

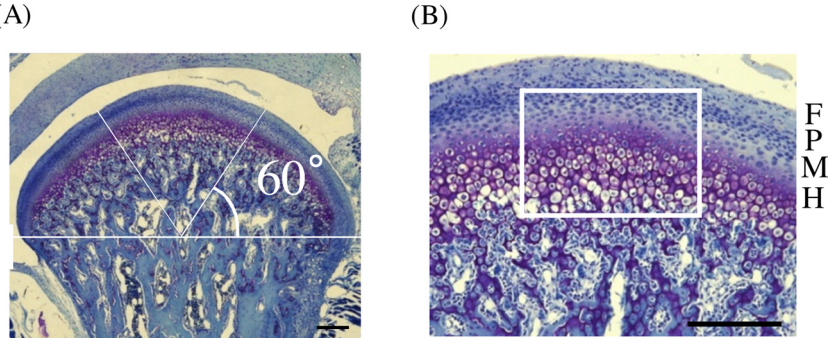

Fig. 1. (A) Frontal section of the mandibular condyle. The upper portion of the condylar head was divided into three regions of 60° each: the lateral, central, and medial regions. Bar = 200 μm. (B) Four layers of cartilage are shown: fibrous layer (F); proliferative cell layer (P); mature cell layer (M) and hypertrophic cell layer (H). The number of asporin and TGF-β1 immunopositive cells was calculated in an area 200 μm × 140 μm in the proliferative and mature layers of the superior region of the condylar cartilage. Bar = 50 μm.