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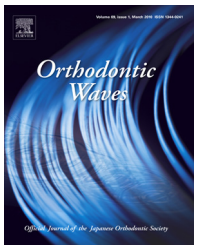

Original article

Unilateral nasal obstruction induces morphological changes of the mandibular condyle in growing rats

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ABSTRACT

Purpose: Chronic nasal obstruction is known to decrease blood oxygen saturation. Mouth breathing in association with chronic nasal obstruction leads to the collapse of the buccinator mechanism and to a clockwise rotation of the mandible, which causes mandibular retrusion. This study aimed to investigate the influences of nasal obstruction on the morphological and histological changes of the mandible in growing rats.

Materials and methods: Thirty 8-day-old male Wistar rats were randomly divided into the control and experimental groups. The experimental group underwent unilateral nasal obstruction by cauterization of the external nostrils at 8days of age. Pulse oxygen saturation (SpO_2) was monitored every week. Rats were sacrificed at 9 weeks of age. The mandibular changes were analyzed via lateral cephalometric radiographs and micro-CT scans. We utilized toluidine blue and tartrate-resistant acid phosphatase (TRAP) staining for histological analysis. Immunohistochemical staining of hypoxia induced factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), osteoprotegerin (OPG) receptor activator of nuclear factor kappa-B ligand (RANKL) were also performed to reveal the mechanism of the morphological changes.

Results: SpO_2 was significantly lower in the experimental than in the control group. In the experimental group, length, bone mineral density and cartilage layer thickness of mandibular condyle were decreased. The number of TRAP-positive cells in the condyle, HIF-1α-positive cells, VEGF-positive cells and RANKL-positive cells in the condylar cartilage was significantly increased. In contrast, a reduced expression of OPG protein was observed in the experimental group.

Conclusions: Our findings suggest that unilateral nasal obstruction in the growth period affects mandibular morphology.

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1. Introduction

Recently, the prevalence of allergic rhinitis has increased worldwide, occurring in 10% to 30% of adults and up to 45% of children [1]. Allergic rhinitis is a representative symptom of nasal respiratory disorder characterized by three major symptoms: sneezing, nasal mucus secretion, and nasal obstruction (NO). Chronic NO is known to decrease pulse oxygen saturation (SpO_2) [2]. Further, lung weight reduction and hormonal behavioral changes have been reported in rat models of bilateral NO [3,4].

Previous studies have demonstrated how mouth breathing associated with rhinostenosis influences the function of the buccinators. Mouth breathing induces lip dysraphism and triggers the clockwise rotation of the mandible further causing mandibular retrusion and Class II malocclusion in humans [5]. Recent studies have revealed a relationship between NO and malocclusion. Mouth breathing combined with nasal congestion induces a vascular mechanism collapse that leads to adenoid facies resulting from the backward rotation of the mandible [6]. This combination has also been found to cause interference of the lip closure, high palate, and Angle Class II malocclusion in humans [7]. Unilateral NO (UNO) was found to suppress the jaw-opening reflex in a rat model [8]. In addition, it has been reported that NO affects not only muscle reflexes but also the growth of both muscle and bone in the rat model [3,9]. In the craniofacial area, decreased growth of the masseter superficial layer and the anterior belly of the digastric muscle, including the vertical reduction of the nasomaxillary complex, were observed in a rat model of bilateral NO [10,11]. In summary, NO has various effects on the craniofacial region; however, the underlying mechanisms of the mandibular morphological changes associated with NO are still unclear.

Hypoxia induced factor-1 αlpha (HIF-1α) expression is associated with the decrease of SpO_2 under hypoxic condition in rabbits [12]. It is activated during hypoxia and involved in osteogenesis and angiogenesis [13]. It was also reported that HIF-1α acts on the vascular endothelial growth factor (VEGF) to promote osteoblastic and osteoclastic differentiation and regulate chondrocyte apoptosis through the glycolysis system in rats [14,15]. In addition, the osteoclast activation mechanism of HIF-1α has previously been reported: hypoxia upregulates the glycolysis pathway and increases the expression of glucose transporters [16]. Subsequently, HIF-1α expression increases in osteoclasts with the release of cathepsin K and hydrogen ions resulting in bone resorption in mice [17].

Osteoprotegerin (OPG) is another protein that is reportedly related to osteoclast activation. And, OPG is downregulated under hypoxia, thus promoting osteoclast activation in rats [18]. OPG acts as the receptor activator of nuclear factor kappa-B ligand (RANKL) decoy receptor in the RANK-RANKL system. Generally, its upregulation suppresses osteoclast activity through binding to RANKL in vitro [19]. An increased RANKL/OPG ratio and a decreased number of OPG-positive cells were detected in the previous study using osteoarthritic TMJ of rats [20].

However, to our knowledge, no study has yet investigated the relation between the chronic hypoxia caused by the NO-related expression of HIF-1α, OPG, RANKL and tartrate-resistant acid phosphatase (TRAP) activity and the reduction in bone volume. In this study, we evaluated the influence of UNO on the mandibular morphology of growing rats through the assessment of the HIF-1α expression, OPG expression, RANKL expression and TRAP activity in the condyle.

2. Materials and methods

2.1. Animal preparation

Animal protocols were approved by the Institutional Animal Care and Use Committee of the Tokyo Medical and Dental University (#0170370A), and the experimental procedures were performed in accordance with the University's Animal Care Standards.

Thirty 8-day-old male Wistar rats were used in this experiment. The rats were randomly divided into the control and experimental groups (n=15 each). All rats were first anesthetized by hypothermia (10 min at −18°C). UNO was induced in the experimental group by cauterization of the external nostril on post-natal day 8. Cauterization was performed by burning the surrounding tissues of the left nostril, using a 400°C-stainless-steel wire that was 1 mm in diameter. For the first week, we visually checked every day whether cauterized noses remained closed; we re-cauterized each nose immediately if it re-opened. After 1 week, we observed cauterized noses once per week and verified that they never opened during the experimental period. The control group underwent a sham operation in which the cauterizing instrument was placed 1–2 mm above the left nostril. 3% chlortetracycline (Aureomycin® Ointment; Pola Pharma, Tokyo, Japan) was applied on the left external nostril of rats in both groups postoperatively to prevent infection [21]. Weight and pulse oxygen saturation were monitored every week using a pulse oximeter (MouseOx® STARR Life Sciences Corp., Oakmont, PA). Both the experimental and control group animals were euthanized at 9 weeks using CO_2 gas.

2.2. Radiological analysis of the mandible and tibia

The radiological analysis was performed using the procedures reported previously [22]. Briefly, lateral radiographs were obtained with a soft X-ray system (SOFTEX CMB-2; SOFTEX Co., Ltd., Tokyo, Japan) to evaluate craniofacial morphological changes. The head of each rat (n=15 each) was fixed using a pair of ear rods to maintain a standard head position. The head was maintained in contact with the film to reduce the magnification factor [23]. The various parts of the mandibular bone were measured with the NIH image software (NIS-Elements Analysis D, National Institutes of Health, Bethesda, MD, USA). The measurement points are shown in Table 1 and Fig. 1. Selected linear measurements were then obtained (Table 2). The cephalometric landmarks (Table 1, Fig. 1) were derived from the previous studies in rodents [24,25]. The soft X-ray settings were 50 kVp, 15 mA, and 5-s impulse [23]. The whole tibia was collected and its length was measured as an indicator of whole body growth. All radiographs were taken three times by the same operator.

2.3. Microcomputed tomography analysis (Micro-CT)

Micro-CT analysis and a desktop X-ray micro-CT system (SMX-100CT; Shimadzu, Kyoto, Japan) were utilized to investigate the

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