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Decreased bone marrow stromal cells activity involves in unilateral anterior crossbite-induced early subchondral bone loss of temporomandibular joints

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ABSTRACT

Objective: Subchondral bone loss in mandibular condyles was reported to be induced by experimentally created unilateral anterior crossbite (UAC) which altered the occlusal load distribution and hereafter the temporomandibular joint (TMJ) remodelling process. However, the initial cellular responses are poorly understood. In the present study, changes in osteoblast and osteoclast activities in TMJ subchondral bone were investigated using the rats treated with UAC.

Design: Forty rats were randomly divided into UAC and control groups, and sampled at 2 weeks after the operation. Subchondral bone loss was evaluated by micro-CT. Osteoclast and osteoblast activities were analyzed by real-time PCR. The osteoblast differentiation of the locally isolated BMSCs from TMJ subchondral bone was assessed by Alizarin red staining. The migration of BMSCs was detected by transwell assays.

Results: Compared with the age-matched controls, TMJ subchondral bone loss was observed in the UAC-treated rats ($p < 0.05$). The osteoblast activity evaluated by real-time PCR and osteoblast number revealed by immunohistochemical staining were reduced in the TMJ subchondral bone of UAC rats ($p < 0.05$), and the capability of proliferation, migration and osteoblast differentiation were all decreased in the locally isolated BMSCs from the UAC group ($p < 0.05$).

Conclusions: The present data demonstrated an involvement of reduced BMSCs activity in the initiation of the mandibular subchondral bone loss at the early stage of installation of the aberrant prostheses.

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Abbreviations: TMD, temporomandibular joint disorders; TMJ, temporomandibular joint; UAC, unilateral anterior crossbite; BMSCs, bone marrow stromal cells; TRAP, tartrate-resistant acid phosphatase.

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1. Introduction

Under most conditions, osteoclast-mediated bone resorption and osteoblast-mediated bone formation are tightly coupled. Bone remodelling, which is completed by both osteoclasts and osteoblasts, continues throughout life and occurs in response to the mechanical balance alterations of the skeleton and its musculature. Bone loss is well known to occur when the amount of bone resorption exceeds that of bone formation.[1] Acute occlusion changes can alter the load distribution on TMJ, resulting in alterations in the joint structures.[2] Previously, we reported a kind of aberrant occlusal relationship in mice that we named as unilateral anterior crossbite (UAC) in which subchondral bone loss was induced.[3] How osteoblast and osteoclast activities engaged in the early stage of this subchondral bone loss worths an investigation.

Mesenchymal stromal cells (MSCs) are the basic cellular unit of embryologic bone formation. MSCs can be easily obtained from the adult bone marrow and contribute to the regeneration of mesenchymal tissues including bone.[4] Bone marrow stromal cells (BMSCs) play an important role in bone healing in adults by migrating to the area of damage following differentiating into osteoblastic lineages.[5] Abundant data conclusively demonstrated that osteoblasts direct osteoclast differentiation.[6–9] It implies that BMSCs may play an important role in bone remodelling.

In this study, using our reported UAC rats, micro-CT analysis was adopted to evaluate the morphological changes of the TMJ subchondral bone. The expression levels of genes related to the activities of osteoclast and osteoblast were examined by real-time PCR. The locally isolated BMSCs from TMJ subchondral bone marrow were examined for proliferation, migration and mineralization. The goal of this study was to observe the initial changes in the activities of BMSCs from the subchondral bone marrow of the UAC rats and their role in the subchondral bone loss induced by UAC.

2. Materials and methods

2.1. Animals and dental operation

Forty female Sprague-Dawley rats (6 weeks old; weighing 140–160 g) were obtained from the animal centre of the Fourth Military Medical University. All animal experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1996) and were approved by the Fourth Military Medical University Animal Care and Use Committee. Rats were randomly divided into the sham-operated control (Con) group and the unilateral anterior crossbite (UAC) group ($n = 20$).

Unilateral anterior crossbite was created as previously described.[10] Briefly, an erect metal tube (inside diameter = 3 mm, length = 2.5 mm; ShinvaAnde, Shangdong, China) and a curved metal tube (inside diameter = 2 mm, length = 4.5 mm; ShinvaAnde, Shangdong, China) with an angle of 135° to the labial side at the upper end were bonded to the left maxillary and mandibular incisor, respectively (Fig. 1). Rats were sacrificed at 2 weeks after the operation.

2.2. Micro-CT analysis

The rats were scanned with an *in vitro* micro-CT system (eXplore Locus SP, GE, Fairfield, CT, US) at 80 kV and 80 μA 2 weeks after the operation ($n = 5$). The X-ray beam was reconstructed with an isotropic voxel size of 8 μm. The value of BV/TV (the ratio of bone volume to tissue volume), Tb.N (trabecular number), Tb.Th (trabecular thickness) and Tb.Sp (trabecular separation) were measured.

2.3. Histochemical and immunohistochemical staining

TMJ blocks were fixed, decalcified, dehydrated and embedded by conventional methods. 5-μm thick sections were used for TRAP and immunohistochemical staining. Sections were stained with tartrate-resistant acid phosphatase (TRAP; Sigma–Aldrich, St. Louis, MO, USA) in accordance with the manufacturer's recommendations. Immunohistochemical staining with anti-osteocalcin primary antibody (1:200, sc-30044, Santa Cruz Biotechnology, Inc., CA, USA) was performed as a standard, three-step, avidin–biotin complex staining procedure. Images were captured by a Leica light microscope (Leica, Solms, Hessen, Germany). The average numbers of TRAP-positive cells and osteocalcin-positive cells per millimetre along the bone marrow cavity were calculated.

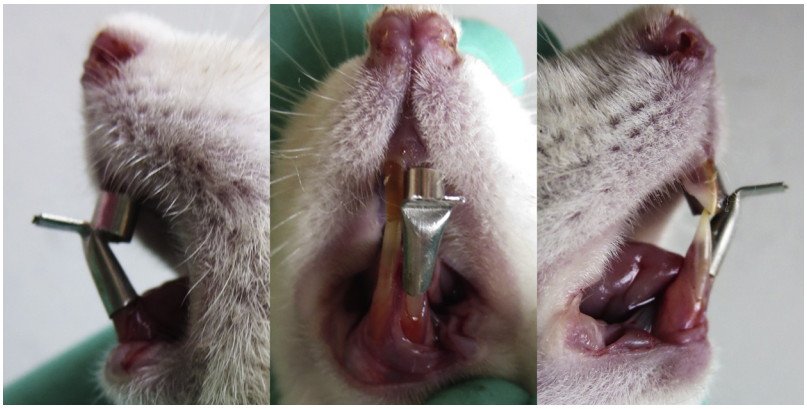

Fig. 1 – Unilateral anterior crossbite was created by bonding metal tubes to the left pair of incisors.