Condylar cartilaginous changes after mandibular distraction osteogenesis in rabbits

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Objective. The purpose of this study was to evaluate histologic and immunohistochemical changes in the condylar articular surface in response to distraction osteogenesis (DO) of the mandibular body in rabbits.

Study design. A unilateral osteotomy of the mandible at the premolar area was performed in 16 rabbits. The animals were divided into 4 groups based on different distraction parameters (rate and rhythm). After a 5-day latency, the mandible was lengthened by 0.5 mm daily for 6 days (group 1); 0.5 mm twice daily for 3 days (group 2); 0.5 mm once daily for 10 days (group 3); or 0.5 mm twice daily for 5 days (group 4). All 4 groups underwent a 14-day consolidation period. Four rabbits were included in the control group. The specimens were stained with hematoxylin-eosin for histologic examination. Immunohistochemical analysis was performed to evaluate the expression of growth factors.

Results. None of the groups demonstrated any degenerative changes in the temporomandibular joint (TMJ). On the distraction side in all groups, the histopathological examination revealed a hypertrophic thickening of the cartilage zone. Prominent endochondral ossification and high active osteoblasts were observed in groups 3 and 4. On the nondistraction side, no major changes were observed excluding the appearance of osteoclasts in groups 3 and 4. The immunohistochemical analysis revealed tenasin immunoreactivity in bone marrow mesenchymal cells on the distraction side in group 4. Connexin immunoreactivity did not display a marked change in any of the groups. Osteocalcin was observed on the distraction side in group 2, which suggested that bone formation is increased. Nitric oxide synthase 2 immunoreactivity was observed on the distraction side in group 2, which is associated with stress and inflammation.

Conclusions. The results indicated that the hypertrophy of the cartilage zone and endochondral ossification became more pronounced as the extent and rate of distraction increased. (Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:416-422)

Distraction osteogenesis (DO) is a surgical process in which 2 bony segments are gradually separated to allow the formation of new soft tissue and bone between them. It is used in the reconstruction of skeletal deformities. After a surgical fracture is achieved by performing either a corticotomy or an osteotomy, a fixation device is used to deliver a gradual lengthening force.1-3 This procedure was first described by Codivilla4 in 1905. Ilizarov5,6 observed the development of bone in the gap between fragments of bone in which only fibrous scar tissue had been present in 1 patient. He concluded that the maintenance of distraction at an appropriate rate induced bone formation, bone lengthening, and bone continuity. The term “tension-stress principle” was coined for this technique, which involves a gradual, controlled displacement of surgically created fractures that result in a simultaneous expansion of soft and hard tissues with an increase in bone volume. Ilizarov5,6 thus established the theoretic basis for the procedure.
The concepts described by Ilizarov have been adapted and modified for use in the oral and maxillofacial field to reconstruct alveolar bone defects. Recently, DO has been used frequently for the reconstruction of congenital or acquired deformities, such as cleft palate and unilateral or bilateral microsomia in the mandible. The effect of DO on the temporomandibular joint (TMJ) when performed on the mandibular body or the mandibular ascending ramus in unilateral microsomia cases has been the focus of numerous studies.

To our knowledge, this is the first immunohistochemical study to assess tenascin, connexin, osteocalcin, and nitric oxide synthase 2 expression in the TMJ after DO.

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**MATERIAL AND METHODS**

The study protocol was approved by the Animal Research Committee of Chosun University Dental Hospital.

**Study population**

Twenty healthy rabbits that weighed 2 to 3 kg were used in this study. Among this group, 16 were divided into 2 experimental groups of 8 animals each (distraction and nondistraction sides). Four animals (TMJs) comprised the control group. The experimental groups were subdivided according to the extent of distraction. The extent of distraction was 3 mm and 5 mm in groups 1 and 2 and in groups 3 and 4, respectively. Distraction was performed at a rate of 0.5 mm daily for 6 days (group 1); 0.5 mm twice daily for 3 days (group 2); 0.5 mm once daily for 10 days (group 3); and 0.5 mm twice daily for 5 days (group 4). Four animals were assigned to each group (Table I) and maintained with standard animal feed under identical conditions.

The DO devices were 1 cm long and 2 cm wide, which was achieved by adding orthodontic self-curing resin to a 5-mm expansion screw (FöresaDent, Pforzheim, Germany) and fixed with an 8-mm self-tapping screw (Jeil, Seoul, Korea).

### Corticotomy and device fixation

Before surgery, the animals received an intramuscular injection of 5 mg/kg gentamicin (Green Cross, Yongin, Korea), the hairs on the left side of the mandibular inferior border were removed, and the overlying skin was sterilized to prevent infection. General anesthesia was induced via an intramuscular injection of 5-mg/kg ketamine HCL (Ketalar, Yoo Han Yang Hang, Seoul, Korea) and 0.3 mg/kg xylazine (Rompun; Bayer, Korea, Seoul, Korea). To reduce bleeding, the animals received an intrarotagonal and extraoral injection of 2% lidocaine HCl (containing 1:100,000 epinephrine). A skin incision was made from the mandibular angle to the cuspid to expose the mandibular inferior border. A vertical osteotomy was performed using a fissure bur in the first premolar area of the mandible with saline irrigation. A device was constructed to serve as the resection surface in the corticotomy area, and it was placed in the center of the segments and fixed to the mandible with self-tapping screws.

The wound was sutured, and gentamicin (5 mg/kg) was administered via an intramuscular injection once daily for 5 days after surgery to prevent infection.

### Latency, distraction, and consolidation periods

The corticotomy and device fixation were followed by a 5-day latency period. A second surgery was performed to expose the distraction device, which was attached to the surgical site and operated via an extraoral incision. Preoperatively, 5 mg/kg gentamicin (Green Cross) and 2% lidocaine HCl (containing 1:100,000 epinephrine) were injected intramuscularly at the surgical site to prevent infection. General anesthesia was induced via an intramuscular injection of 5 mg/kg ketamine HCl (Ketalar, Yoo Han Yang Hang) and a venous injection of 3-mg/kg xylazine (Rompun; Bayer, Korea). The distraction device was exposed by opening the sutured area. Sutures were placed on both sides of the distraction device using black silk to expose the expansion screw of the distraction device.

After the second surgery, distraction was performed according to the following protocol: group 1 to 0.5 mm once daily for 6 days (total, 3 mm); group 2 to 0.5 mm twice daily for 3 days (total, 3 mm); group 3 to 0.5 mm once daily for 10 days (total, 5 mm); and group 4 to 0.5 mm twice daily for 5 days (total, 5 mm). All 4 groups were allowed a consolidation period of 14 days.

### Preparation of tissue specimens

After the latency period (5 days), distraction period (6, 3, 10, and 5 days), and consolidation period (14 days).
days), the rabbits were humanely killed, and a tissue block containing condyle and disk was harvested. The specimens had the following dimensions: 0.8 × 0.8 × 0.8 cm. Half of the specimens were prepared as decalcified samples and examined under a light microscope, and the other half were prepared for immunohistochemical staining.

To prepare the decalcified samples, the tissues were maintained in 10% formalin for 2 weeks and subsequently decalcified with nitric acid (de-cal Rapid; Pational Diagnosis, Atlanta, GA) for 24 hours, embedded in paraffin according to conventional methods, sectioned in the sagittal plane to a thickness of 5 \( \mu \text{m} \), and stained with hematoxylin and eosin (H&E).

For immunohistochemistry, paraffin-embedded blocks were sectioned to a thickness of 5 \( \mu \text{m} \), mounted on 2% 3-aminopropyltriethoxy-silane–coated slides, deparaffinized, and dehydrated. The sections were incubated in phosphate-buffered saline (PBS) solution containing 1% bovine serum albumin for 30 minutes and treated with PBS containing 0.01% protease type XXIV (Sigma, St. Louis, MO) at room temperature for 10 minutes. After washing with PBS, the sections were incubated with rabbit antihuman tenasin (1:50; Serotec, Oxford, UK), rabbit anticonnexin (1:100, Zymed Laboratory, San Francisco, CA), goat anti-osteocalcin (1:100; Santa Cruz Biotechnology, Santa Cruz, CA), and goat anti-NOS2 (1:100; Santa Cruz Biotechnology) at 4°C. After washing with PBS, the tissues were incubated with biotinylated goat antirabbit immunoglobulin (IgG antibody (1:200). The samples were subsequently incubated with 0.1 M Tris-HCl buffer (pH 7.2).

Fifty tissue sections were prepared from 1 tissue block. Five sections each were used for H&E and immunohistochemistry.

**RESULTS**

**Light microscopic findings**

*Control group.* The condylar surface was covered with an avascular fibrous connective tissue layer that contained some cartilage with collagen fibers running in parallel to the condylar surface. Beneath the condylar surface, a fibrous articular, proliferative, hypertrophic cartilage zone and subarticular bone were observed (Fig. 1).

![Fig. 1. Photomicrograph of the control group showing a normal condyle (H&E stain, original magnification ×40). Fl, fibrous articular layer; Hcz, hypertrophic cartilage zone; Pz, proliferative zone.](image1)

![Fig. 2. Photomicrograph of the distraction side in Group 1 after treatment. The Hcz thickened, and the number of chondrocytes (arrows) increased (H&E stain, original magnification ×100). D, disk; Hcz, hypertrophic cartilage zone.](image2)

![Fig. 3. Photomicrograph of the distraction side in Group 2 after surgery. The hypertrophic cartilage zone thickened, the number of chondrocytes increased, and osteoblasts appeared (arrows) (H&E stain, original magnification ×40).](image3)
Group 1. An increased amount of cartilage in the proliferative zone, thickening of the fibrous articular and hypertrophic cartilaginous cell layers, and cartilaginous ossification were observed. An increased number of osteoblasts in the subchondral bone was also detected (Fig. 2).

Group 2. An increased number of cartilage cells in the proliferative zone and thickening of the fibrous articular and hypertrophic cartilaginous cell layers were observed. Cartilaginous ossification was also detected. However, on the nondistraction side, ossification did not occur, and the number of osteoclasts did not increase (Fig. 3).

Group 3. The number of cartilage cells in the proliferative zone increased to a level that was comparable with that in groups 1 and 2. The fibrous articular layer demonstrated an increase in thickness, and cartilaginous ossification was observed (Fig. 4).

Group 4. An increased number of cartilage cells in the proliferative zone and thickening of the fibrous articular layer were observed. In addition, enhanced...
cartilagenous ossification was evident in the subchondral bone (Fig. 5).

Immunohistochemical results

Immunohistochemical reactivity to tenasin was observed in group 4, which implied that the number of cartilage cells had increased in this group. In addition, gap junction–forming connexin was not expressed in the bone and cartilage. On the distraction side in group 2, the expression of osteocalcin was increased (Fig. 6), which is associated with bone formation and is expressed in response to osteoblastic activity, and the relative expression of nitric oxide synthase 2, which is associated with stress and inflammatory reactions (Fig. 7).

DISCUSSION

Several diverse methods have long been used to reconstruct congenital or acquired bone defects or deficiencies. The representative methods are bone grafting using autologous or heterologous bone, the insertion of bone substitute materials, and flap surgery. Potential problems that are associated with these procedures are the resorption of the grafted bone, infection, new defects in the donor site, or an insufficient or rapid changes in the surrounding soft tissues. DO compensates for such disadvantages because additional surgery of the donor site is not required, and distraction is achieved simultaneously with the development of adjacent tissues. In addition, the problems associated with rapid changes in the adjacent tissues, the deficiency of soft tissue, and limitations concerning the length of the graft area are ameliorated.

DO is a technique that restores bone defects that are caused by trauma, tumors, and congenital deformities via induction of the biological healing process of the bone. Clinically, DO is performed in 4 stages. First, an osteotomy is performed in the area that requires distraction, and a distraction device is implanted. Before performing the distraction, a latency period of a predetermined length is established to allow formation of the primary callus, similar to the fracture-healing process. If the latency period is too short, a fibrous union or nonunion may develop instead of the primary callus; if it is too long, the osteotomy area will undergo ossification.8,9 The latency period allows the formation of a soft callus, which stimulates the formation of new tissues by delivering the distraction to tissues located between the resected bone segments. The distraction is performed after the latency period. The consolidation period occurs between completion of the distraction and removal of the distraction device. At this time, the bones that are newly formed after distraction mature and obtain strength. The promising features of this procedure in comparison with the limitations of traditional bone grafting have spurred continuous scientific investigations regarding its applications. The use of DO in the oral and maxillofacial area has been studied and reported changes in the newly formed bone tissues, muscles, nerves, and TMJ.

Mandibular microsomia can result from congenital deformities, trauma, rheumatoid arthritis, radiotherapy, and tumor resection. However, DO has certain advantages in comparison with the costal graft. For example, lengthening of the mandibular bone does not cause discomfort or impairment of the donor site. It can be performed in cases in which the costal graft has failed or the patients do not desire a bone graft. In addition, it can overcome the limitations that are caused by changes in the vertical, horizontal, or anteroposterior length.10 The shortcomings of DO are as follows: it may cause malocclusion and infection or TMJ impairment. To compensate for these shortcomings, it is essential to develop appropriate presurgical plans, perform an accurate evaluation of the patients, and control the distraction device in a precise direction.11

The greatest challenge associated with mandibular distraction may be that the changes in the TMJ caused by distraction cannot be considered separately, as demonstrated in the studies conducted to date.

McCarthy et al.12 described mandibular distraction using extraoral devices in humans and showed that the mandible was successfully lengthened by the application of bone distraction in 4 unilateral microsomia patients. Kulewicz et al.13 demonstrated that the performance of unilateral mandibular distraction was effective in 2- to 15-year-old growing patients; however, they asserted that appropriate corrective treatments were required during the consolidation period. Hamada et al.14 demonstrated the retrusion of a section of the condylar area with no major changes in the condyle. In addition, Kusnoto et al.15 reported that unilateral mandibular distraction in growing unilateral microsomia patients did not lead to any major problems with respect to their growth. Gabbay et al.16 studied transport distraction osteogenesis and Matthews Device arthroplasty in TMJ bony ankylosis with hemifacial microsomia patients and demonstrated that both were successful initially. However, the Matthews Device arthroplasty avoided long-term relapse. Ko et al.17 treated growing patients with unilateral distraction and reported stable results. Similarly, Shetye et al.18 found no major problems in growing patients with mandibular microsomia who underwent a unilateral distraction.

Harper et al.19 described the bone distraction of the mandibular midline and observed thickening of the fibrous layer in the TMJ and cartilage layer. Furthermore, the osteoblast reactivity was observed to increase...
and subsequently return to baseline levels. Copray et al.20,21 reported that a small, continuous force delivered to the condyle stimulated proliferation of the subchondral cell layer, which was reduced by intermittent force. For matrix formation, the subchondral cell layer is proliferative during the delivery of intermittent force. Nakai et al.22 reported that an intermittent compression force stimulates ossification within cartilage and that this reaction facilitates the growth potential of condylar cartilage. In addition, they noted that condylar cartilage was resistant to external change and induced stability in the growing mandibular condyle. Samchukov et al.23 stated that during the application of the stress analysis in distraction, the rotation of the condyle axis was induced by the lateral rotation of the mandible and distraction of the midline of the mandible; thus, the device must be implanted parallel to the distraction side.

Kofod et al.24,25 studied the stress induced in the TMJ area during unilateral mandibular distraction in patients with arthritis. He found that stress was delivered to the posterior side of the nondistraction side; however, a partial increase in the force was observed. Nevertheless, a low level of stress was detected in the TMJ area. A simulation showed that the stress was more centralized during unilateral distraction and became identical to that detected on the nondistraction side. Kim et al.26 examined the mandible in rabbits during unilateral mandibular distraction and did not detect degenerative or inflammatory changes, although endochondral ossification was observed on the distraction side.

Thurmuller et al.27 examined the TMJ in pigs after mandibular distraction and found that the medial articular disk had become thinner. An increased distraction rate (4 mm/d) resulted in degenerative or inflammatory changes in the condyle and articular disk; however, such changes were not evident when the distraction rate was reduced (1 mm/d). Zou et al.28 studied the distraction rate and changes in the mandibular joint and found that a distraction rate of 1 mm/d resulted in inflammatory changes in the condyle and cartilage; however, a distraction rate of 2 mm/d caused the induction of degenerative changes. Liu et al.29 reported that excessive increases in the rate of distraction led to degenerative or inflammatory alterations of the condyle and cartilage in white rats.

In the present study, changes in the condyle were examined in relation to the rate and extent of distraction during unilateral mandibular distraction using light microscopy and immunohistochemistry. Equivalent histologic and immunohistochemical findings were obtained for all of the animals. The results revealed an increase in the number of cartilage cells in the proliferative layer on the distraction side and an increased thickness of the hypertrophic cartilage layer. An increased rate and extent of distraction was associated with a greater number of cartilage cells. Immunohistochemically, degenerative changes in the TMJ were not detected in response to an increased rate and extent of distraction.

However, because the rabbits were killed immediately after the consolidation period, the development of long-term changes on the distraction side and nondistraction side could not be evaluated. The use of a restricted distraction of up to 5 mm places further limitations on the findings of the present study.

CONCLUSIONS

Degenerative changes in the TMJ were not detected during a unilateral mandibular distraction in rabbits. An increased extent of distraction resulted in elevated expression levels of osteocalcin on the distraction side because of an increased number of osteoblasts. As the rate of distraction was increased, the expression of nitric oxide synthase 2 was observed in response to the stress and inflammatory reaction. Degenerative changes in the TMJ that are dependent on the extent and rate of distraction were not detected during the unilateral mandibular distraction.

We recommend that future studies focus on greater increases in the extent and rate of distraction to evaluate their effects on the condyle with respect to the time lapse after the consolidation period.

REFERENCES


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