An Animal Model for Inducing Anterior Disc Displacement of the Temporomandibular Joint

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Aims: *To develop an animal model of anterior disc displacement (ADD) without the need for opening the temporomandibular joint (TMJ) capsule.* ***Methods:*** *Thirty-two healthy adult Japanese white rabbits were used in this study. Four rabbits were dissected to familiarize the investigators with the anatomy of the TMJ. Sixteen animals were subjected to surgical ADD in the right TMJ, 8 animals had a sham operation, and 4 animals were not operated (normal controls). Four rabbits from the experimental ADD group and 2 from the sham group were sacrificed 1, 2, 4, and 8 weeks, respectively. The rabbits in normal control group were sacrificed at the beginning of the experiment. Animal behaviors as well as macro- and microchanges in the TMJs were investigated.* ***Results:*** *Fifteen right TMJ discs in the 16 experimental rabbits were successfully displaced anteriorly, and the degree of ADD in the experimental group was similar. The mandible of each ADD rabbit deviated to the left side with inclined attrition of the incisors. Some histologic changes appeared in the experimental TMJs.* ***Conclusion:*** *This ADD technique without the need for opening the TMJ capsule is effective, and the model is suitable for studying ADD of the TMJ.* J OROFAC PAIN 2006;20:166–173

Key words: animal model, anterior disc displacement, rabbits, temporomandibular joint

Disc displacement of the temporomandibular joint (TMJ) and its associated pathologic and clinical consequences, which may include pain, have been extensively studied; however, there are still many unresolved aspects about its progress and mechanisms.[1–5] Although x-ray, computed tomography, and magnetic resonance imaging can be performed clinically on humans, specific histologic examination is usually only possible on cadavers or dissection specimens. The clinical history of disc displacement is usually unknown for cadavers. Clinical history is usually known for specimens donated for dissection, but they are usually obtained from patients who were subjected to various clinical interventions, which complicates interpretation of the observations.[6] Moreover, the sources of these materials are limited and generally lack controls. Thus, a valid animal model of anterior disc displacement (ADD) is necessary for investigating TMJ changes after ADD.

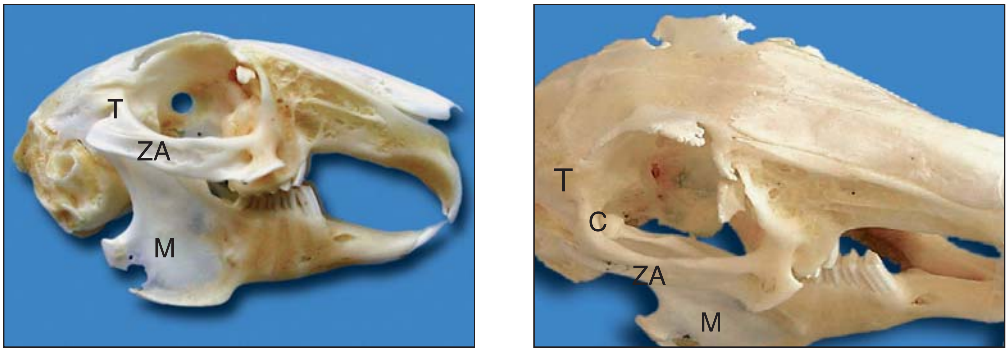

Fig 1 Bone structure of rabbit TMJ. C = condyle, T = temporal bone, M = mandible, ZA = zygomatic arch.

Because several of its structural and functional characteristics are comparable to humans, the rabbit is a suitable animal to use to establish an ADD model for studying disc displacement of the TMJ.[6–9] Over the past 20 years, many researchers have established ADD rabbit models.[6,7,10–12] Most researchers have established their models[6,7,10] by opening the TMJ capsule and damaging the attachment of the disc. Some[11,12] have opened the capsule and retained the traction suture in the joint space while keeping the attachment of the disc intact. The traction suture may constantly rub the surface of the disc and cause secondary injury. In the animal model of Imai et al, the disc was anteriorly displaced relative to the condyle by moving the condyle superioposteriorly.[13] The relationship between the disc and the articular eminence was not changed. Therefore, these models do not correspond to the usual occurrence of disc displacement observed in humans.

The aim of this study was to develop a new model of ADD in which the capsule and attachments remained intact, without the need to open the TMJ capsule.

Materials and Methods

Experimental Animals and Groups

Thirty-two healthy adult Japanese white rabbits (weight, 2.5 to 3 kg) provided by the Medical Animal Center of Zhejiang University were used in the study. The animal experimental protocol was approved by the Ethical Committee on Animal Experiments of the Hospital of Stomatology, Zhejiang University. Four rabbits were dissected to familiarize the investigators with the TMJ and its surrounding tissues. The remaining 28 rabbits were divided into 3 groups: 16 in the experimental group, 8 in the sham group and 4 in the reference group. The rabbits in the experimental group were subjected to surgical ADD in the right TMJ, and 4 rabbits were sacrificed at 1, 2, 4, and 8 weeks, respectively, after surgery. In the sham group, the right TMJs were operated with same procedure as in the experimental group, but the discs were not anteriorly displaced. Two animals from the sham group were killed at 1, 2, 4, and 8 weeks, respectively, after surgery. The animals in the reference group had no operation but were sacrificed at the beginning of the experiment and served as normal controls.

Surgical Technique

The rabbits were anesthetized by intravenous injection of sodium amobarbital (3 mL/kg) via an ear vein. The hair in the preauricular region was shaved unilaterally. The surgical site was sterilized and draped in preparation for surgery, and the surgical region was locally anesthetized with 2% lidocaine (1.5 mL). A curvilinear incision about 2.5 to 3 cm (with the concave side facing inferiorly) was made along the zygomatic arch, from just superior to the temporal root of the zygomatic arch to just inferior to the inner lateral canthus of the eye. Then the skin and periosteum were reflected to expose the zygomatic arch, which constituted the inferior wall of the orbit and which comprised the zygomatic process of the temporal bone and temporal process of the zygoma (Fig 1). There were 2 small processes at the superior border of the anterior third of the zygomatic arch. A hole was drilled through just anteroinferior to the front process, and a stainless-steel wire 0.25 mm in diameter was pushed through. Thereafter, fat tissue in the orbit

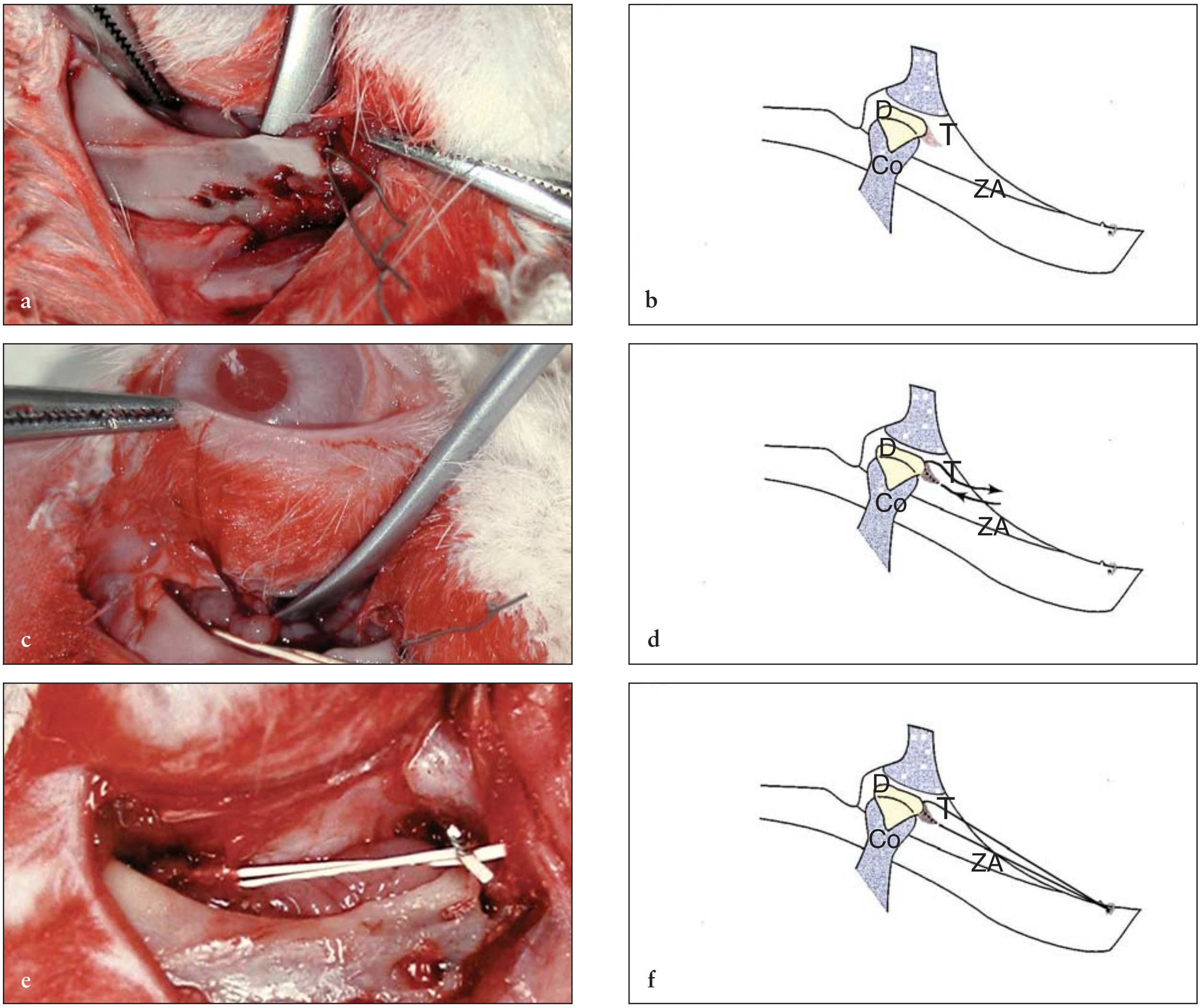

Fig 2 The surgical process of creating the ADD. Photographs of the lateral view of the right TMJ during surgery are shown beside sketches of the surgical process. The zygomatosquamosal process covers the disc-condyle complex and the articular tubercle. *(a and b)* The zygomatic arch was exposed, and a hole was drilled just anterioinferior to the front process, which was located at the inferior wall of the orbit. A stainless-steel wire (0.25 mm diameter) was pierced through. *(c and d)* A round needle with an elastic rubber band was passed from the orbital cavity to the anterior extension of the disc inferiorly and then pulled into the orbital cavity. *(e and f)* The disc was stretched forward by double strands of the band. The other end of the band was fixed to the zygomatic arch by the stainless-steel wire. The rubber band was stretched from a starting length of 7 mm to a final length of 16 mm. It could produce a force of about 85 g. Co = condyle; ZA = zygomatic arch; D = disc; T = temporal bone.

was pushed aside to expose the posterior wall of the orbit. Care was taken to avoid lancinating the anterior temporal attachment of the disc. A round needle with an elastic rubber band (made for orthodontic purposes by TP Orthodontics) was passed vertically through the anterior extension of the disc. The disc was stretched forward by double strands of the band, and the other end of the band was fixed to the zygomatic arch by a stainless-steel wire (Fig 2). The 7-mm rubber band was stretched to a length of 16 mm before being fixed, producing a pulling strength of about 85 g, which was determined by a stress and tension gauge (BIOM Hangzhou Westlake Biomaterial).

When these procedures were completed, the wound was thoroughly irrigated by saline. The surgical site was then closed in a layered fashion, and the rabbits were returned to their cages, where they were provided with rabbit chow ad libitum and water. The same methods were used for the sham operations, except for the forward traction on the disc.

Specimen Preparation

In all animals, general anesthesia was induced before the animals were sacrificed with an air embolus. Careful dissection was performed down to the joint level, and all joints were opened and checked grossly to determine the condition of the displacement. The characteristics of the joints were also observed. After this gross examination, the TMJs were removed en bloc and fixed in 10% neutral buffered formalin for 24 hours. The samples were demineralized in 10% EDTA for 4 to 6 weeks. Each sample was sectioned with a razor blade in the sagittal plane, and the tissue block was embedded in paraffin. Serial sections of 5 μm thickness were cut and stained with hematoxylin-eosin (H&E).

Fig 3 Tooth abrasion in an experimental ADD rabbit. Front view of an ADD rabbit (8 weeks after surgery). Note the obliquely abraded incisors.

Results

Animal Behaviors and Gross Appearance of the TMJs

All experimental and sham-operated animals tolerated the surgical procedures well. Except for light edema a few days after surgery on the lower eyelid, there were no significant postoperative problems. All animals gained weight during the experiment, and there was no significant difference in weight between the reference and experimental groups. Normal mastication and patterns of occlusal activity (gnawing of cage bars) had recovered by 3 to 5 days after the operation. The mandibles of all ADD-induced animals deviated to the left side to some extent, with inclined attrition of the incisors (Fig 3). The longer the ADD developed, the more obvious the deviation of the mandible and the attrition of the incisors became.

Gross observation showed that the anterior band of the disc was located directly beneath the crest of the articular eminence in the jaw-closed state in the sham and reference groups. The posterior band was just above the superior crest of the condyle.

Displacement of the disc was judged successful if the posterior band was located anterior to the superior crest of the condyle in the jaw-closed state. With this criterion, 15 of 16 discs in the experimental group were anteriorly displaced. The degree of ADD was similar in these 15 animals. In a 1-week experimental rabbit, the disc was notably less displaced because of a broken rubber band, which might have been caused by the clamps on the rubber band. This specimen was removed from the experimental group.

Compared with the reference group, the sham-operative specimens showed no noteworthy differences in disc position, gross shape, or histologic structure. Hence, the term "control" used hereafter refers to the reference and sham groups.

Anatomy of Rabbit TMJ

The rabbit TMJ specifically involved articulation with the articular tubercle and the mandibular fossa of the temporal bone and with the head of the mandible. The disc completely divided the TMJ into superior and inferior compartments. The articular fossa was immediately medial to the root of the zygomatic arch of the temporal bone as it passed anteriorly to articulate with the zygoma. Two small processes were seen anteroposteriorly at the superior border of the inferior wall of the orbit. The anterior one, which was relatively stable, was located at the anterior third of the inferior wall of orbit. It was about 16 mm from the temporal root of zygomatic arch to the anterior process at the inferior wall of the orbit (see Fig 1). The inferoposterior part of the orbital cavity was composed of fat tissue, which could be easily pushed aside to expose the posterior area of the orbit, where the anterior temporal attachment of the disc extended as far as the anterior edge of the articular tubercle, and inferiorly the inferior anterior attachment of the disc attached to the neck of the mandible. From the inferoposterior part of the orbit, the anterior extension of the disc could easily be approached.

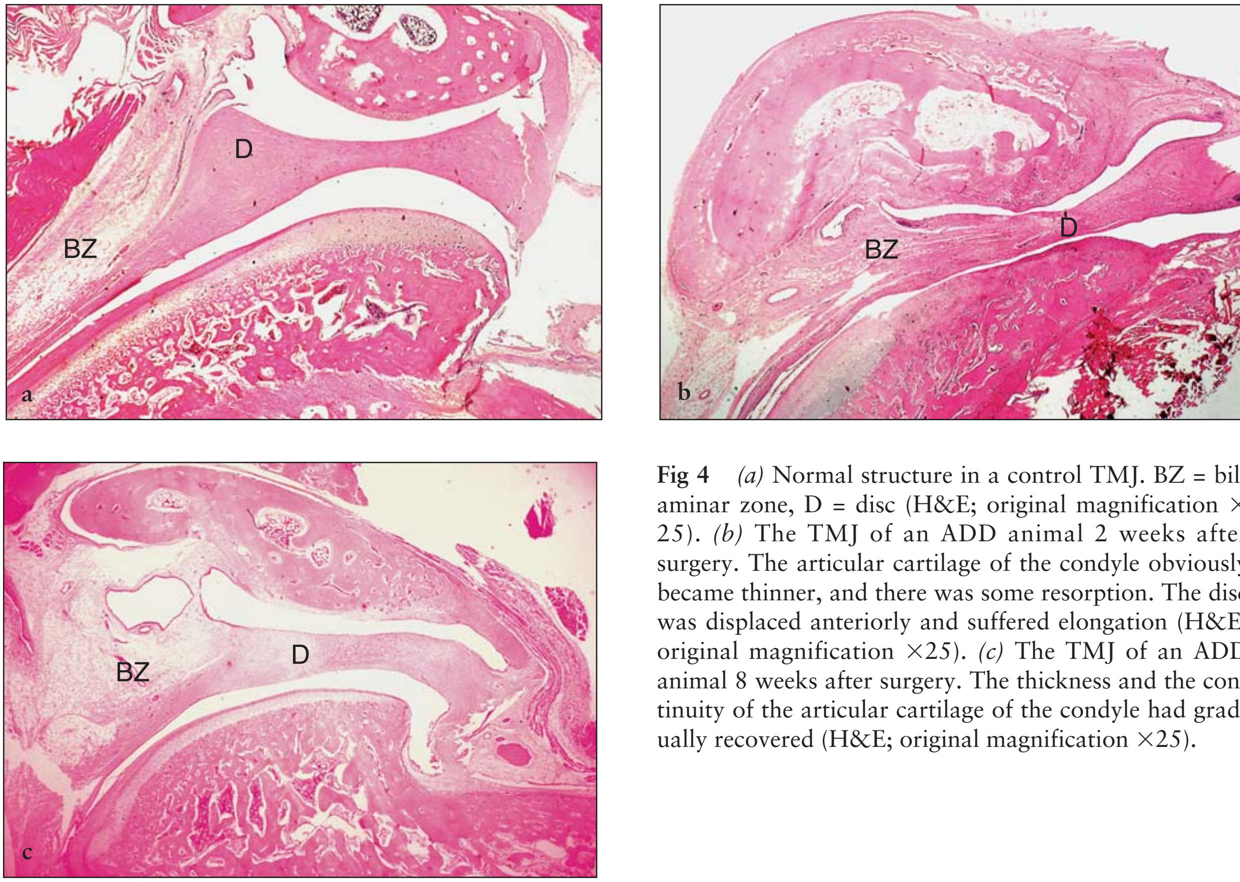

Fig 4 *(a)* Normal structure in a control TMJ. BZ = bilaminar zone, D = disc (H&E; original magnification × 25). *(b)* The TMJ of an ADD animal 2 weeks after surgery. The articular cartilage of the condyle obviously became thinner, and there was some resorption. The disc was displaced anteriorly and suffered elongation (H&E; original magnification ×25). *(c)* The TMJ of an ADD animal 8 weeks after surgery. The thickness and the continuity of the articular cartilage of the condyle had gradually recovered (H&E; original magnification ×25).

Microscopic Observations

Condyle. One week after ADD, the condylar cartilage had become thinner, and some surfaces were damaged. In 1 specimen from the 2-week group, humplike changes could be found in the condylar cartilage, which appeared to be have the histopathologic characteristics of osteoarthropathy. The articular cartilage of the condyle recovered gradually in the 4 weeks after surgery. Normal articular cartilage was seen in most 8-week specimens (Figs 4a to 4c). Similar changes were also found in the cartilage of the articular eminence.

Disc. The control discs were biconcave and comprised a thick anterior band, a thin intermediate zone, and a thick posterior band. The posterior band was just over the crest of the mandibular condyle (Figs 4a and 4b). There were many round chondrocytes in the disc, with round or oval nuclei, and a few fibro-chondrocytes. In the experimental TMJs, all discs suffered elongation, deformation, and obvious anterior displacement. No fragmentation or perforation was found in the anteriorly displaced discs after surgery.

Bilaminar Zone. The bilaminar zone (BZ) was composed of loose fibrous fatty connective tissue, rich in collagenous and elastic fibers. Most cells in the BZ were shuttle-shaped fibroblasts with long shuttle-shaped nuclei (Fig 5a). After ADD, the density of fibrous tissue and fibroblast in superior attachment increased gradually. The walls of blood vessels in the inferior attachment also thickened, leading to a narrower cavity. Some chondrocyte-like cells appeared (Fig 5b). In a rabbit of the 8-week group, puerile chondroid metaplasia was found in the junction of the posterior band and the BZ (Fig 5c).

No morphological changes of articular tissue occurred in the sham-operated group.

Discussion

ADD is the most frequent type of internal derangement. Internal derangement is considered to be related to the pathogenesis of TMJ-related pain

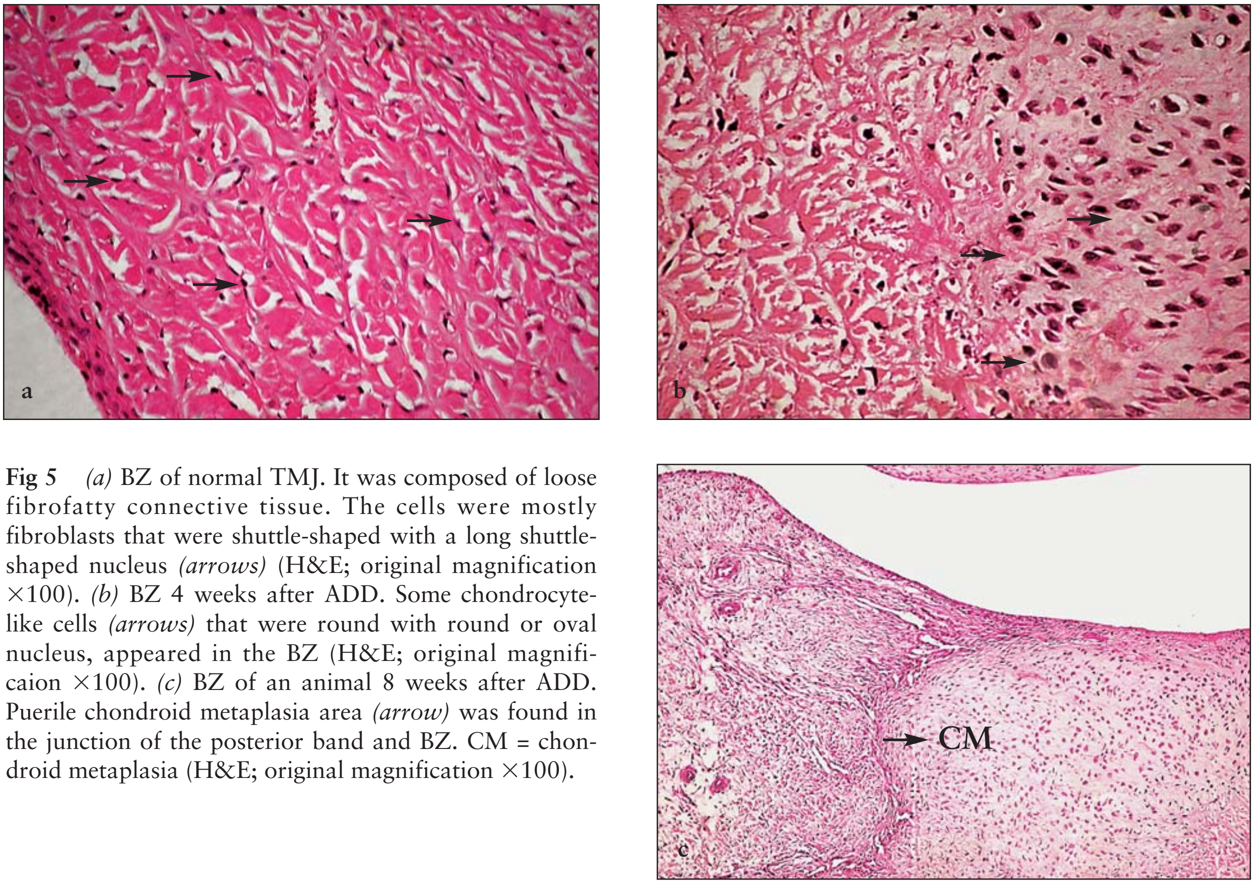

Fig 5 *(a)* BZ of normal TMJ. It was composed of loose fibrofatty connective tissue. The cells were mostly fibroblasts that were shuttle-shaped with a long shuttle-shaped nucleus *(arrows)* (H&E; original magnification ×100). *(b)* BZ 4 weeks after ADD. Some chondrocyte-like cells *(arrows)* that were round with round or oval nucleus, appeared in the BZ (H&E; original magnificaion ×100). *(c)* BZ of an animal 8 weeks after ADD. Puerile chondroid metaplasia area *(arrow)* was found in the junction of the posterior band and BZ. CM = chondroid metaplasia (H&E; original magnification ×100).

and dysfunction.[14] To study the pathological changes and progress of internal derangement of the TMJ, many animal models of ADD have been established. One rabbit model was established by transection of the temporal and condylar parts of the posterior attachment along their entire mediolateral extent and then displacement of the disc anteriorly between the condyle and eminence.[7] Another rabbit model was established by fracturing the zygomatic process of the squamosal bone and pulling the disc forward with a suture fixed to the zygomatic arch and cutting the anterior medial and lateral disc attachment.[8] The method was improved by Mills et al[6]; they transected the posterior, medial, and lateral attachments of the disc to the squamosal bone, leaving the anterior squamosal attachment and all of the condylar disc attachment intact. These methods, however, damaged the internal structure and function of TMJ and the disc was abruptly induced anteriorly. It would be unlikely for human disc displacement to arise in such an abrupt extreme manner. Although Mills et al[6] noted that it was important to keep part of the disc attachment intact and make the displacement moderately mobile, mobility was limited because the suture was not elastic. Finally, Berteretche et al[12] proposed another animal model in which the disc is pulled forward by a spring device attached by a suture, which would allow the disc to be slightly mobile after ADD. However, the tension placed on the disc was difficult to control. Nevertheless, as the lateral part of the disc was submitted to a tension oriented forward and downward, the disc rotated in the axial plane around its medial attachment and was more anteriorly displaced laterally and medially. In addition, although the attachment was kept intact, the capsule was damaged by the surgery.

In the method used here, the disc was stretched forward with double strands of elastic rubber band that passed through the anterior extension of the disc and fixed to the front process of the inferior wall of the orbit. It was not necessary to remove the surrounding bone and incise the capsule and the attachments. Compared to previous methods, this surgery was much less traumatic to the internal constitution, and it kept the TMJ intact. The fixed point of the elastic rubber band and the tension placed on the anterior extension of the disc by the rubber bands from 7 mm to 16 mm was relatively steady. Consequently, the degree of disc displacement in the experimental group was similar in each subgroup, indicating that the degree of displacement by this method could be easily controlled and was suitable for studying derangement in different periods of ADD. Furthermore, compared to previous methods, in which the ADD was abruptly developed with a thread,[6,7,10,11] in the present study the disc was pulled forward gradually by the elastic rubber band, which approximated the development of ADD in humans. This might be the reason that perforation was not found in the anteriorly displaced disc or BZ in this study but did occur in some other ADD animal models.[6]

In the animal model described by Berteretche et al,[12] the disc was anteriorly displaced by a tension of 1 N on the lateral part of the disc. In their model, the capsule was not incised, and the rubber band was only passed through the anterior extension of the disc, which was easily torn off. In a pilot experiment by the present authors, damage was found in the soft tissue of the TMJ when the pull force produced by the rubber band was larger than 90 g. Thus 85 g of force was used in the present experiment to pull the disc forward. As a result, 15 discs from the 16 experimental rabbits were displaced. This means that the force of 85 g on the anterior extension of the disc was adequate to pull the disc forward. In addition, because the direction of traction was more horizontal in this model, the disc was hardly rotated, and the degree of ADD was relatively similar laterally and medially.

The TMJ is sensitive to mechanical stimuli.[15,16] The histologic changes after ADD observed in the present study also suggest that there is a relationship between the tissue response and the mechanical stimulus. The rubber bands caused the elongation and deformation of the discs as well as ADD. The condyle was also affected in some cases. In the early stage, the condylar cartilage became thinner and had some absorption. However, the cartilage recovered gradually in the 4 weeks following surgery. Therefore, the TMJ has remarkable adaptive potential and self-repair capabilities. If the destruction did not exceed the restoration ability of the TMJ, these destructive changes may have been reversible. As the ADD proceeded and the loading increased, histologic changes in the BZ were also observed in the present study. These included thickened blood vessels, dense collagen fibers, and chondrocyte-like cells. All these changes might suggest that after ADD, with the stimulation of stress on the anteriorly displaced BZ, some remodeling occurred in BZ and made it function as a disc. Such adaptive changes would also be consistent with findings of anteriorly displaced discs in humans following surgical procedures or in autopsy material of older people.[17–21]

The remarkable adaptive abilities observed in the present study may help explain why, in some studies, more than 30 percent of subjects in whom evidence of ADD was found with arthrography or magnetic resonance imaging of TMJs were free from signs and symptoms such as pain.[22–24]

Deviation of the mandible has previously been reported in clinical and animal experimental studies of unilateral ADD.[25–27] Asymmetry of the mandible with or without inclined attrition of the incisors has been attributed to a reduction of mandibular height due to growth disturbances in the mandibular ramus or loss of the condyle caused by degenerative change or osteoarthritis.[25,26,28–31] In the present study, all rabbits subjected to experimental ADD were adult, and no growth disturbance was found in the mandible. Furthermore, because the TMJ capsule was not broken in these rabbits, little damage to the TMJ and no obvious loss of condyle caused by osteoarthritis were observed. A dramatic difference from the ADD model established by others was the finding that the experimental rabbits in this study experienced mandibular deviation to the nonsurgical side, with inclined attrition of the incisors. Anatomically, the capsule was superiorly attached to the periphery of the articular fossa, while anteriorly it extended as far as the anterior edge of the articular tubercle; inferiorly, the capsule attached all around the neck of the mandible. These features ensured that it moved with the mandible during protraction and retraction. The capsule was not opened, and the tension was placed on the anterior extension of the disc. The deviation to the nonsurgical side suggested that the force placed on the anterior extension of disc also pulled the condyle forward.

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