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Induction of traumatic temporomandibular joint ankylosis in growing rats: a preliminary experimental study

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Abstract – There are many reports on the management of pediatric temporomandibular joint (TMJ) ankylosis. However, few authors have investigated the etiology of this disease in children. The purpose of this study was to determine the role of damage to both the condylar cartilage and disk in the induction of traumatic TMJ ankylosis during the growth period. The study was performed in growing rats by a common condyle fracture model. Intentional damage was performed to both the disk and condylar cartilage in the experimental group ($n = 12$), while the disk and condylar cartilage in the control group was left untouched ($n = 12$). Sham-operated growing rats were deemed the blank group ($n = 10$). Two rats from the experimental group and two from the control group were killed 24 h after the operation, and the result following surgical intervention was observed. Each rat's body weight in the three groups was monitored and recorded at 1, 2, 4, 6, 8, 10, and 12 weeks after surgery. Twelve weeks after the operation, all animals were killed. The prognosis was compared by mandible deviation measure, body weight evaluation, and histological observation. Animals from the experimental group presented a slow body weight increase and obvious mandible deviation while all involved TMJs showed fibrous ankylosis in various degrees. The damage to both the condylar cartilage and disk in the condylar fracture might play a vital role in traumatic TMJ ankylosis development during the growth period. The results of this study suggest that more attention should be paid to condylar fractures in children that are accompanied with severe cartilage and disk damage, which is a matter of significance for pediatric TMJ ankylosis prevention.

Most temporomandibular joint (TMJ) ankylosis cases are associated with TMJ trauma resulting from condylar fracture. TMJ ankylosis occurs primarily in the first 10 years of life; onset rates less than 10 years old has been reported between 35% and 92% in all cases (1, 2). There have been many reports on pediatric TMJ ankylosis management (3–8), yet few authors have investigated the etiology in children. In the past, a reproducible adult animal TMJ ankylosis model was reported (1, 9–15). However, to our knowledge, there has yet to be a definite TMJ ankylosis model reported in growing animals.

More rarely following facial trauma joint ankylosis may occur, and there has been considerable speculation as to why few injured joints ankylose and most do not (16). Experiments in adult animals have demonstrated that damage to the condylar cartilage and disectomy appear to be important in TMJ ankylosis development, in which severe articular damage and disk removal resulted in fibrous and bony ankylosis after 3 months (1, 9–14). In addition, if the joint is either immobilized or bone grafted, more extensive fibrous and bony ankylosis will occur (9–14). Child condylar cartilage, the major growing site in the condyle, has more proliferation ability potential than the adult condylar cartilage. Osteogenesis in children is active because the mandible is still in its development. Therefore, osteogenesis in the fracture site might result in condyle hyperplasia. The inartificial disk indicates an important biomechanical role for normal joint function and prevents bony hyperplasia between the condyle and temporal bone. Therefore, we presume that in TMJ trauma caused by condylar fracture, the damage to both condylar cartilage and the disk might be the major contributor to TMJ ankylosis development in children. To prove this presumption, we performed the present experimental study in growing rats using a common condyle fracture model that included intentional damage to both the disk and condylar cartilage.

Materials and methods

Thirty-four 1-month-old male Wistar rats with an average body weight of 71.14 g were used in the experiment. They were randomly separated into three groups (experimental group, $n = 12$; control group, $n = 12$; sham-operated group, $n = 10$). Both research

protocol and animal use in the experiment were approved by the Ethics Committee of the College and Hospital of Stomatology at Wuhan University.

Anesthesia was administered by means of a ketamine hydrochloride intramuscular injection. Each right preauricular area was shaved and prepared with an antiseptic solution. An approximate 10 mm preauricular skin incision was made directly below the right zygomatic arch. Blunt dissection was performed through the masseter muscle. The condylar process was then exposed. In the experimental group, the associated disk attachments over the condylar head were first damaged using eye scissors. The condylar articular surface was then scraped by a probe to cause damage in the condylar cartilage. Condylar cartilage debris was left in the joint. Finally, a subcondylar process fracture was created using mosquito forceps, which was confirmed by condyle fragment mobility. The condyle fragment was then displaced in the medial direction and the wound was closed. For the control group, both the disk over the condylar head and the condylar cartilage was untouched, while a similar unilateral medially displaced condyle fracture was created. The sham-operated animals were submitted to the same procedure but were limited in condylar periosteum exposure. Two random rats in the experimental group and two in the control group were killed 24 h after surgery, which were then subjected to histological observation. The other 30 rats were administered intramuscular penicillin injections during the first 3 days after operation.

All the 30 rats were killed 12 weeks after surgery. During the 12 weeks, each rat's body weight was monitored and recorded at 1, 2, 4, 6, 8, 10, and 12 weeks after operation. The Student's *t*-test was used to evaluate significant difference between the mean body weight values of both groups at each time point. *P*-values less than 0.05 were deemed statistically significant.

After the rats were killed the entire skulls were obtained and fixed in 4% paraformaldehyde for 48 h. The skulls were then radiographed to evaluate the degree of deviation of the mandible, according to the methods described by Luz and de Araujo (17). In brief, the skull was placed directly on a radiographic film, with the inferior border of the mandible as the horizontal plane reference. Radiograms were taken directly from above. Mandible deviation angular measurements were performed by image analysis software (SPOT Version 3.5 for Windows, Diagnostic Instruments, Inc, Sterling Heights, MI, USA) with reference to a line between both tympanic antrum (angle α, Fig. 1). To evaluate the significance between the mean angle α values, the Student's *t*-test was used and the level of significance set at $P < 0.05$.

Specimens from the right TMJ were then obtained, decalcified, dehydrated, and embedded in paraffin for histological observation by Mallory's trichrome stain. All specimens were cut in the coronal plane.

Results

The mean and standard deviation (SD) of body weights for the three groups at each time point are given in Table 1. The animals in the sham-operated group continued to grow and increase in weight. Control group weights decreased after the first week and increased from the second week on. There was a decrease in the experimental group after the first week, and then the weights slowly increased from the second week on but a severe deficiency was noted throughout the experimental period.

Statistical evaluation between mean body weight values of the experimental and sham-operated groups at each time point revealed significant difference at all time points. There was a significant difference between

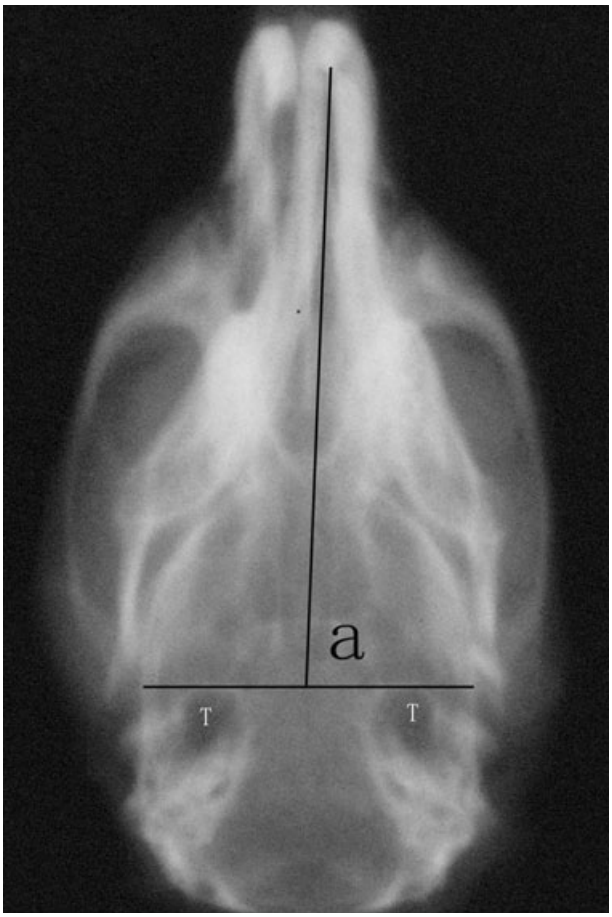

Fig. 1. Radiogram of fixed skull and angle α measurement (vertical line: mandibular midline; T: tympanic bulla).

Table 1. Mean and SD for body weights of the rats

Time point (week)	Experimental group (g)	Control group (g)	Sham-operated group (g)
0	70.66 ± 3.56	70.48 ± 3.51	70.38 ± 3.15
1	60.39 ± 3.18	62.15 ± 4.99	100.21 ± 4.33
2	102.11 ± 7.98	123.21 ± 6.56	134.18 ± 6.46
4	117.43 ± 10.92	172.38 ± 11.55	180.83 ± 8.42
6	142.66 ± 15.02	201.62 ± 12.34	223.74 ± 10.72
8	160.18 ± 20.11	249.85 ± 18.96	256.39 ± 15.37
10	180.73 ± 21.36	276.54 ± 18.91	280.53 ± 18.75
12	185.39 ± 20.68	295.74 ± 22.33	315.49 ± 20.17

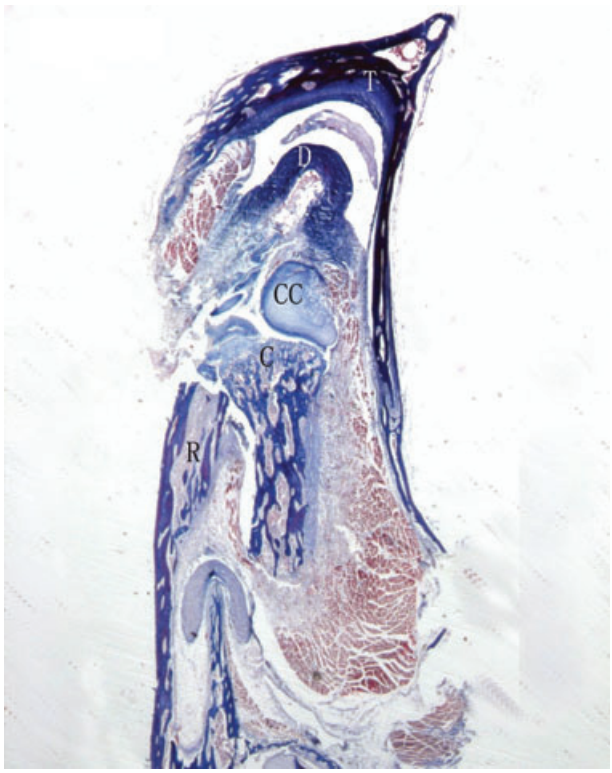

Fig. 2. Temporomandibular joint appearance of the experimental group 24 h after surgical intervention (Mallory's trichrome stain, ×2.0; T: temporal bone; D: disk; CC: condylar cartilage; R: ramus of mandible).

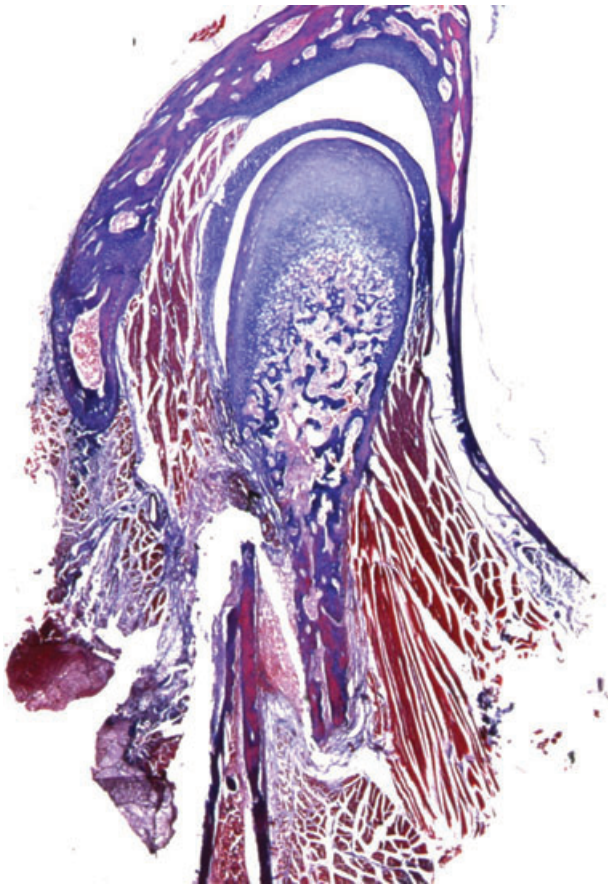

Fig. 3. Temporomandibular joint appearance of the control group 24 h after surgical intervention (Mallory's trichrome stain, ×2.0).

the control and sham-operated groups at the first, second, and fourth week, and no difference at the sixth, eighth, tenth, and twelfth week. There was a significant difference between the experimental and control groups following the first week after surgery.

The mean angle α values in the experimental group was 84.94 ± 0.25, 88.66 ± 0.44 for the control group, and 88.91 ± 0.35 for the sham-operated group. Statistical difference was found between the experimental and control group mean values, as well as the experimental and sham-operated groups. However, no significance was found between the control group and sham-operated group mean values.

Temporomandibular joint appearance 24 h after the surgical intervention is shown in Figs 2 and 3, in which the condylar process fracture with medial displacement is confirmed in both the experimental and control group animals. For the experimental group, the condylar cartilage dissociated from the condyle head. In addition, there was serofibrinous exudation in the joint space. Twelve weeks after the surgery, all TMJs in the experimental group showed over-proliferative fibrocartilage-like tissue surrounding the condyle head, and the joint space either decreased or almost disappeared (Fig. 4). However, in the control group the condyle fracture prognosis was favorable, presenting normal TMJ characteristics. The condylar process was centralized in the temporal fossa and the fracture area was indistinct (Fig. 5). None of the animals in the sham-operated group displayed abnormal histopathological change in the articular and condylar tissue.

Discussion

Clinical investigations show that children had a better outcome of condylar fractures than adults and teenagers, and in children the remodeling of the condylar process was more extensive than that of adults and teenagers (18, 19). The actual incidence of traumatic TMJ ankylosis in children is very infrequent and probably only 0.4% of the cases suffered from condyle fracture (20). However, it cannot be neglected that the onset of TMJ ankylosis is more likely in children than in adults. Unfortunately, TMJ ankylosis studies focusing on the etiological factors during periods of growth are scant. Oztan et al. (21) analyzed the role of different trauma types in TMJ ankylosis formation in growing guinea pigs. It was revealed that mechanical damage to the articular surface and resection of the condylar head almost always resulted in ankylosis. It then seems that mechanical damage to the articular surface and resection of the condylar head might be important dynamics in TMJ ankylosis development during the growing stages. However, the role of a damaged disk can not be neglected during this period

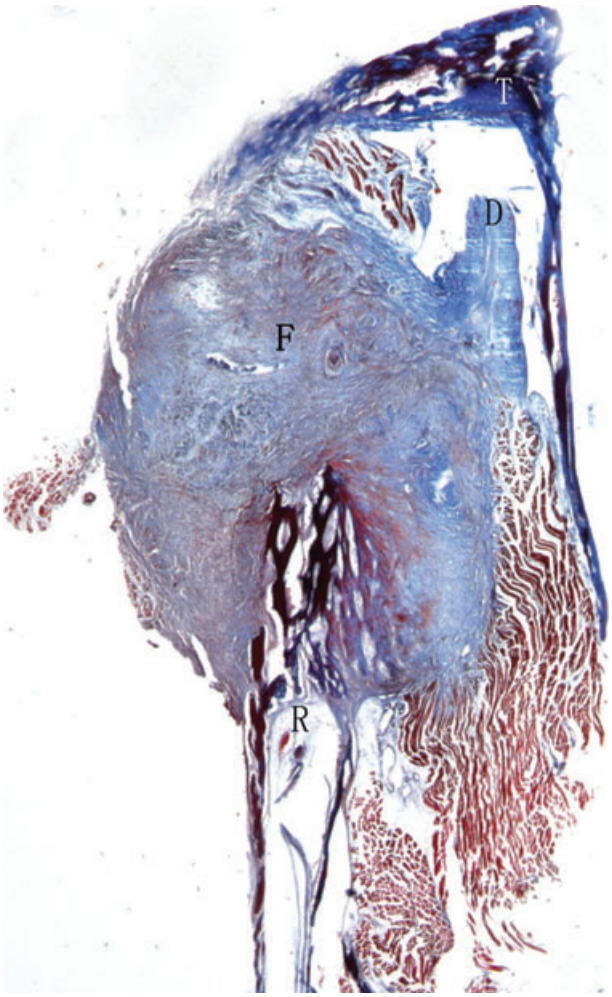

Fig. 4. Temporomandibular joint appearance of the experimental group 12 weeks after the operation (Mallory's trichrome stain, ×2.0; T: temporal bone; D: disk; F: fibrous mass; R: ramus of mandible).

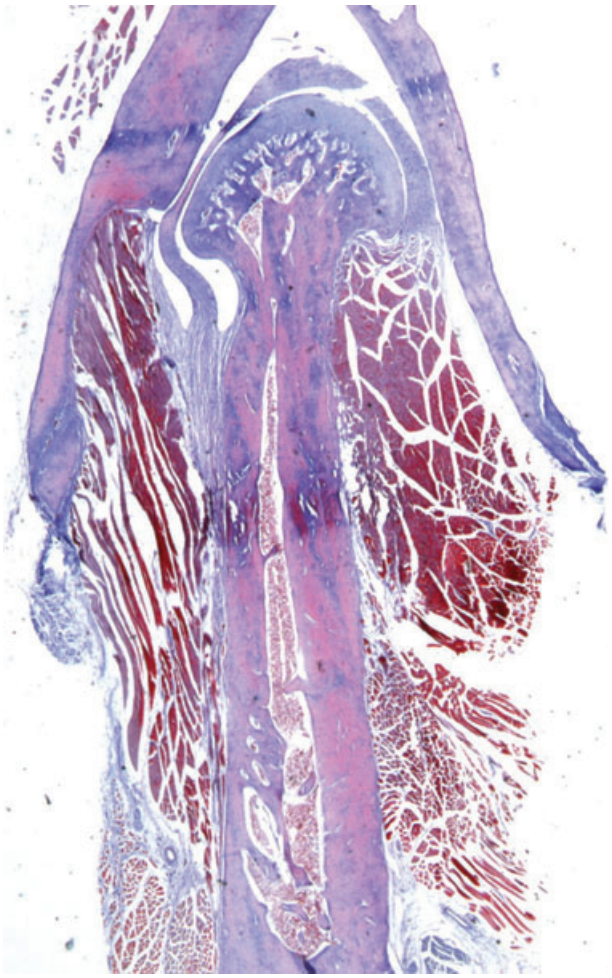

Fig. 5. Temporomandibular joint appearance of the control group 12 weeks after the operation (Mallory's trichrome stain, ×2.0).

as well. Goss and Bosanquet (16) investigated the arthroscopic appearance of acute TMJ trauma and found that hemarthrosis with shredding of the disk and joint surfaces was the most common finding.

The present study showed that subcondylar process fracture combined with intentional damage to the disk and condylar cartilage could result in fibrous TMJ ankylosis in growing rats. The rat is selected as the experimental animal because the growing condyle is similar in structure to that of humans and most other mammals (22–24). Many studies on TMJ trauma obtained convincing results by rat models (17, 25, 26). In the present study, the experimental group rats presented a slow increase in weight and obvious mandible deviation, suggesting a disturbance in both eating and mandible development after the surgical intervention. Such situations are in accordance with clinical cases of pediatric TMJ ankylosis. Furthermore, histological observation results in the experimental group indicated the formation of TMJ ankylosis. In this preliminary experimental study, we did not measure the maximal incisal opening and excursions, which are believed to be the most intuitional evidence in TMJ ankylosis diagnosis. To measure the maximal incisal opening and excursions in an animal experiment the animal must first be anesthetized; then the maximal incisal opening and excursions will be passive. Therefore, it cannot provide true information regarding mandible motion range. In this study, we measured the mandible deviation by objective evidence (angle α). Obviously, once the maximal incisal opening and excursions were measured under anesthesia it would interfere with the angle α measurements.

As for the TMJ trauma animal model in this present experiment, we adopted a unilateral medial displaced fracture, which is the most frequent form of mandibular condyle fracture in the clinic (20, 27). We then advanced it with intentional damage to both the condylar cartilage and disk. However, it does not necessarily mean that this condylar fracture form has close ties with TMJ ankylosis. To date there is no definitive opinion about which condylar fracture form has the closest relation to TMJ

ankylosis. We adopt this condylar fracture model because this fracture type alone cannot cause TMJ ankylosis, which has been verified by other experiments (17, 28). In the present study, we also investigated the prognosis of unilateral medially displaced fracture without intentional damage to the condylar cartilage and disk (control group), and TMJ ankylosis was not observed. Therefore, the experimental results based on this model will be accurate in judging the role of condylar cartilage and disk damage in TMJ ankylosis formation.

In the TMJ trauma animal model in the study, the associated disk attachments over the condylar head were first damaged using eye scissors, but the damaged disk was not removed from the joint. This is different from the adult sheep TMJ ankylosis model (1, 9–15), where the disk was actually removed. In our opinion, the damaged disk should remain in the joint during the natural development of TMJ ankylosis, which has been verified in previous TMJ ankylosis cases (7).

Direct or indirect trauma to the TMJ might cause several injuries such as fibrous adhesion and ankylosis (29). As far as TMJ ankylosis development during the growing period is concerned, condyle cartilage damage should be given more attention. During the growing stages, condylar cartilage is the development center of the condylar process, which has more potential ability of proliferation under an acute condition such as trauma. Once the condylar cartilage is damaged in the condylar fracture, the remnant cartilage overgrowth will be stimulated. With the ossification of over-proliferative fibrocartilage-like tissue, TMJ bony ankylosis will ultimately form. Based on the results from the present preliminary study, we conclude that damage to the condylar cartilage and disk following condylar fracture should be vital causes for the development of TMJ ankylosis during the growing period. The study results also suggest that we should pay more attention to condylar fractures in children accompanied with severe cartilage and disk damage for preventing pediatric TMJ ankylosis. However, the factors related to traumatic TMJ ankylosis development are complex. Hence, more clinic observation is necessary to finally confirm the pivotal role of disk and articular cartilage damage in traumatic TMJ ankylosis development during periods of growth.

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