An Animal Model for Inducing Deviation of the Mandible

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Purpose: Altering the occlusal surface is still a common choice for inducing a deviated mandible in an animal model. Botulinum neurotoxin type A (BoTx/A) can block the action potential transmission in neuromuscular junctions by inhibiting acetylcholine release without damaging the nerves and muscle structures. Our present study was aimed at developing an easy-to-reproduce animal model of asymmetric mandibles in which injection of BoTx/A was applied.

Materials and Methods: A total of 96 healthy 4-week-old male Sprague-Dawley rats were divided into 2 groups: an experimental group (n = 48) with BoTx/A injection and a control group (n = 48) with sterile saline injection at 4 sites of the right masseter muscle. Twelve rats from each group were humanely euthanized at weeks 1, 2, 3, and 4 for morphometric analysis using the micro-computed tomography (CT) findings.

Results: The micro-CT scans revealed facial asymmetry in the experimental group, with no facial asymmetry in the control group after injection. Significant differences were found between the experimental and control groups regarding the indexes containing the mandibular length (length from condyle to menton, length from coronoid to menton, and length of mandibular corpus from gonion to menton) and ramus height (posterior border and middle region near coronoid, and height of anterior mandible at vertical distance from menton).

Conclusion: Our data have indicated that this deviated mandible animal model induced by injection of BoTx/A is highly reproducible and might be proved suitable for future studies of the asymmetric mandible.

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Symmetric, balanced, and harmonious proportions contribute to facial beauty.[1] For orthodontists, facial asymmetry, including midline deviation and lateral malocclusion, is one of the most complex situations to treat. Orthognathic surgery can be required for a more acceptable result when the problems are beyond the scope of orthodontic treatment alone.

It has generally been believed that mandibular asymmetry results from imbalanced lateral and vertical growth of maxillofacial structures. For instance, asymmetric endochondral bone formation in mandibular condylar cartilage can be induced by unilateral temporomandibular joint disorders (TMDs) and osteoarthritis during the growth period, followed by imbalanced growth of the mandibular ramus. In contrast, overgrowth of the condyle, leading to elongation of the mandibular ramus, can also induce mandibular asymmetry.[2] A valid animal model of the deviated mandible is necessary to study mandibular asymmetry further.

During the past 30 years, many efforts have been made to develop an animal model of mandibular asymmetry. One approach was to weaken the mandibular muscles unilaterally by electrolytic lesioning of the motor neurons to these muscles.[3,4] Another

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approach was to alter the occlusal surfaces such that the animal had to shift its mandible in 1 direction during closing.[5-11] The third approach was to injure the articular disk by removal or displacement during the growth period of the animal. However, these methods were not easy to reproduce. Furthermore, the modeling procedures that involved muscle motor neuron lesions, an intraoral positioner appliance, or surgical damage to the articular disk proved to have issues such as peripheral tissue damage, a considerable incidence of displacement of the intraoral positioner appliance, and the consequent functional deficit of the disk.[3,12-14]

In the present study, we used botulinum neurotoxin type A (BoTx/A) to develop an asymmetric mandible model. BoTx/A blocks the action potential transmission in neuromuscular junctions by inhibiting acetylcholine release without damaging the nerves and muscle structures.[15] The use of BoTx/A has proved effective in the management of sialorrhea, TMD, bruxism, focal dystonia, muscle spasm, and masseter muscle hypertrophy.[16-20] Many practitioners have believed that simply paralyzing the muscle will also improve the bony flaring.[21] However, the mechanism is not yet clear. Micro-computed tomography (CT) has been shown to be efficient for small animal imaging.[22] In mandibular evaluation regarding the mechanism of how the masseter muscle affects the mandible, the method of measurement by Nakano et al[23] has been used by pertinent studies[24,25] and was, therefore, also applied in the present study.

The aim of the present study was to develop an easy-to-reproduce animal model of an asymmetric mandible using injection of BoTx/A. We hypothesized that an injection of BoTx/A into the right side of the masseter muscle would induce a deviated mandible.

Materials and Methods

EXPERIMENTAL ANIMALS AND GROUPS

A total of 96 healthy 4-week-old, male Sprague-Dawley rats purchased from the Medical Animal Center of Ninth People's Hospital affiliated to Shanghai Jiao Tong University were used in our study. The study followed the Declaration of Helsinki on the medical protocol and ethics, and the regional ethical review board of Ninth People's Hospital affiliated to Shanghai Jiao Tong University approved the present study. The 96 rats were randomly divided into 2 groups: 48 in the experimental group and 48 in the control group. The rats in the experimental group were subjected to BoTx/A (Allergan, Inc, Toronto, Ontario, Canada) injection into the right masseter muscle, and the control group received saline injections. Twelve rats from each group were humanely euthanized at weeks 1, 2, 3, and 4 for morphometric analysis.

SURGICAL TECHNIQUE

The experimental rats were anesthetized with 10% (vol/vol) chloral hydrate (0.35 mg/kg intraperitoneally). The hair in the parotideomasseteric region was shaved unilaterally. The surgical site was sterilized and draped in preparation for the BoTx/A. A curvilinear incision about 2.5 to 3 cm (with the concave side facing inferiorly) was made along the mandibular ramus to expose the masseter muscle. Four injection sites were located (Figs 1 to 5). According to our previous study,[26-28] BoTx/A was diluted in 0.9% saline solution to reach a working concentration of 2.5 U/mL. The masseter muscles were injected with a total volume of 400 μL Botx/A at the 4 sites. The injection contained 1.0 U BoTx/A. After surgery, all rats received buprenorphine 0.05 mg/kg. The surgical site was closed in a layered fashion, and the rats were returned to the animal room until they awoke. They were provided with rat chow and water ad libitum. The same procedure was used for the control group for the sterile saline injections.

MICRO-CT ANALYSIS OF BONY SYMMETRY

The skulls of the rats were evaluated using the GE eXplore Locus Small Animal MicroCT Scanner (GE Healthcare, London, Ontario, Canada) and a 45-μm voxel protocol with the following scan parameters: 80 kV, 450 μA, and 400 ms exposure time. Sagittal cross images were taken, followed by 3-dimensional (3D) reconstruction using GE Microview, version

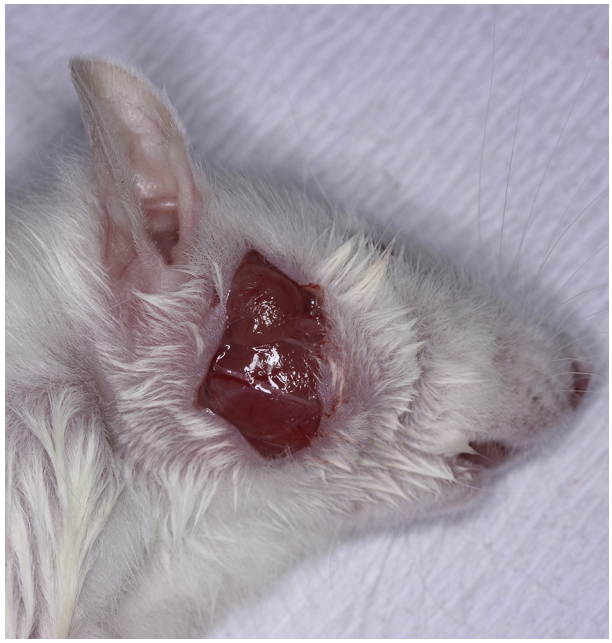

FIGURE 1. Injection sites of a total of 1 U of botulinum neurotoxin type A and the saline vehicle. The masseter muscle was exposed.

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