Oral Diseases (2008) 14, 626–632. doi:10.1111/j.1601-0825.2007.01435.x
© 2008 The Authors. Journal compilation © 2008 Blackwell Munksgaard
All rights reserved
http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Growth effects of botulinum toxin type A injected into masseter muscle on a developing rat mandible

J-Y Kim[1]*, S-T Kim[2]*, S-W Cho[3], H-S Jung[3], K-T Park[1], H-K Son[4]

[1]*Department of Pediatric Dentistry, The Institute of Oral Health Science, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea;* [2]*TMJ & Orofacial pain clinic, Department of Oral Medicine, College of Dentistry, Yonsei University, Seoul, Korea;* [3]*Division in Anatomy and Developmental Biology, Department of Oral Biology, Research Center for Orofacial Hard Tissue Regeneration, Brain Korea 21 Project, Oral Science Research Center, College of Dentistry, Yonsei Center of Biotechnology, Yonsei University, Seoul, Korea;* [4]*Department of Pediatric Dentistry, College of Dentistry, Yonsei University, Seoul, Korea*

OBJECTIVES: Botulinum toxin type A (BTX-A) reduces the muscular contractions by temporarily inhibiting the release of acetylcholine at the neuromuscular junction. The purpose of this study was to investigate the effects of the BTX-A injected into the masseter muscle of a developing rat mandible.

MATERIALS AND METHODS: Four-week-old male (no. 80) Sprague–Dawley rats were divided into four groups: control group, saline group, BTX-A group and baseline control group. Rats of baseline group were sacrificed at 0 day to provide baseline values of the mandibular measurements. The masseter muscle of rats in the saline and the BTX-A group were administered with saline and BTX-A solutions respectively. Experimental animals were sacrificed after 4 weeks.

RESULTS: The BTX-A group demonstrated smaller mandibular dimension compared with the other groups ($P < 0.05$). Their condylar cartilages showed increased apoptosis at the proliferation stage of the reserve zone and masseter muscle fibers demonstrated atrophic changes.

CONCLUSIONS: The result demonstrated BTX-A influence on inhibitory action of the developing mandible because of apoptosis at the proliferation stage of the reserve zone of the condylar cartilage in developing rat mandible.

Oral Diseases (2008) **14**, 626–632

Keywords: botulinum toxin type A; masseter muscle; rat mandible; condylar cartilage

Correspondence: Heung-Kyu Son, DDS, PhD, Professor, Department of Pediatric dentistry, College of Dentistry, Yonsei University, 134 Shinchon-Dong, Seodaemun-Gu, Seoul 120-749, Korea. Tel: 82 2 2123 2015, Fax: 82 2 364 1078, E-mail: hgson@yumc.yonsei.ac.kr
Received 12 July 2007; revised 7 October 2007; accepted 13 November 2007
*These authors contributed equally to this work.

Introduction

Botulinum toxin type A (BTX-A) is a paralytic neurotoxin produced by *Clostridium botulinum*, a causative agent in food-borne botulism (Pearce *et al*, 1994; Jankovic and Brin, 1997; Carruthers and Carruthers, 2001). BTX-A binds specifically to the cholinergic motor end-plate and is actively taken up by motor neurons. It blocks the release of acetylcholine and causes presynaptic neuromuscular blockade (Moore and Wood, 1994; Niamtu, 1999). When highly diluted unit dose of BTX-A is injected into the striated muscles, the toxin produces a reproducible temporary state of regional denervation (Borodic and Pearce, 1994; Smyth, 1994).

As the first use of BTX-A in the treatment of strabismus, BTX-A had proved to be effective in the treatment of disorders characterized by localized muscle hyperactivity (Scott *et al*, 1989; Borodic and Pearce, 1994; Smyth, 1994; Jankovic and Brin, 1997). Furthermore, BTX-A has been applied to treat rhytids for cosmetic purposes, tension- and migraine headaches, and focal hyperhidrosis (Jankovic and Brin, 1997; Carruthers and Carruthers, 1998, 2001; Niamtu, 1999). The BTX-A can be used for almost any conditions in which a reduction in the amount of contracting muscle or glandular secretion is desired.

In dentistry, BTX-A is used for the treatment of masseteric hypertrophy. It gives rise to semi-permanent, transient decrease of muscle volume. As a result, parafunctional habit is reduced along with cosmetic improvement (Moore and Wood, 1994; Smyth, 1994; Freund *et al*, 1999; Mandel and Tharakan, 1999; To *et al*, 2001; Von Lindern *et al*, 2001). In addition, BTX-A injection provides an effective treatment for severe bruxism-related

neurological disorders (Ivanhoe *et al*, 1997; Tan and Jankovic, 2000). As the masseter muscle is closely related to the mandibular growth, clinicians should consider the effect of BTX-A on the mandibular growth when injecting it into masseter muscle of the growing patients. It has long been thought that bony processes are dependent on normal muscle function for their development and maintenance (Nakata, 1981; Hinton, 1991).

The purpose of this study was to determine the effects of the BTX-A injected into the masseter muscle of a growing rat mandible. For this purpose, the overall growth alteration in the mandibular measurements was evaluated in the control, saline injection, and BTX-A injection groups. In addition, the relationship between the histologic changes in the condylar cartilage and gross changes in the mandibles was examined. Histologic changes in masseter muscle following BTX-A injection were also evaluated.

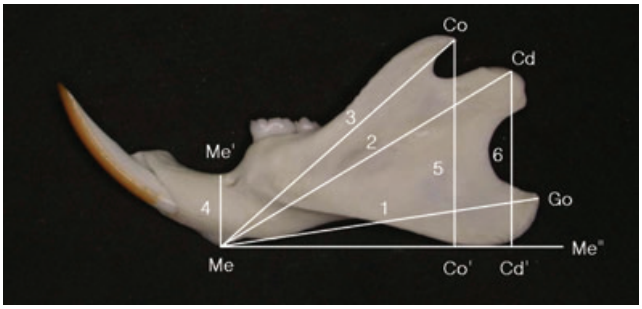

Figure 1 Landmarks and measurements of the mandible used in this study. Me, most inferior point of mental protuberance; Me′, most inferior point of anterior alveolar bone; Me′-Me″, tangent to the bottom of angular process through Me; Go, most posterior tip of the angular process; Cd, the central point of the condyle; Cd′, crossing point on Me-Me″ perpendicular to Me-Me″ from Cd; Co, tip of coronoid process; Co′, crossing point on Me-Me″ perpendicular to Me-Me″ from Co; Me-Go, mandibular body length; Me-Cd, condylar length; Me-Co, coronoid process length; Me-Me′, anterior region height; Co-Co', coronoid process height; Cd-Cd', condylar height

Materials and methods

All experiments were performed according to the guidelines of the Intramural Animal Use and Care Committee, College of Dentistry, Yonsei University.

Experimental set 1

Subjects and study design

Four-week-old male (no. 80) Sprague–Dawley rats were randomly divided into four groups. The body weight of each rat was measured. One group of rats was sacrificed at the beginning of the experiment to provide baseline values of mandibular measurements (baseline control group). The remaining three groups were designated as control group, saline group and BTX-A group respectively.

The masseter muscle of rats in saline- and BTX-A group were injected with saline and BTX-A solutions, respectively. The BTX-A used in this study was BTXA® (Lanzhou Institute of Biological Products, Lanzhou, China). One vial of BTXA® (100 U) was diluted with 2 ml of saline. 0.05 ml (2.5 U) of BTXA® solution for the BTX-A group, 0.05 ml of saline for the saline group were administered on both sides of superficial portion of the masseter muscle. All subjects were weighed weekly and were sacrificed after 4 weeks.

Dry mandible

Fifteen rats in each experimental group and 20 rats of baseline control group were prepared for dry mandibles and the mandible bone itself was separated into the two halves. Photographs of dry mandibles were taken using digital camera, which was heldat a fixed distance from the mandible and transferred to a computer image. The mandibular measurements used in this study were adopted from Dr Asano's study (1986) (Figure 1).

Histological study

Five rats in each experimental group were fixed by perfusion with 4% paraformaldehyde (PFA) solution. The decapitated, skinned heads were fixed in 4% PFA in phosphate buffered saline (PBS) at 4°C. Temporomandibular joint (TMJ) area was resected and decalcified with 10% ethylenediaminetetraacetic acid for 4 weeks at 4°C. The specimens were embedded in paraffin. Serial sagittal sections were made at 7 μm thickness. The section was stained with hematoxylin and eosin (H-E).

The other sections were blocked in 3% hydrogen peroxide for 15 min. The tissue sections were boiled in 10 mM citrate buffer (pH 6.0) for 10 min and cooled at room temperature for 20 min. The slides were incubated in mouse monoclonal antibody against proliferation cell nuclear antigen (PCNA) antibodies (Cat. No. MS-106; Lab Vision, Fremont, CA, USA) at 4°C overnight. After washing with PBS and incubated the biotinylated goat anti-mouse secondary antibody and streptavidin peroxidase at room temperature for 10 min each. Finally, the specimens were visualized using a diaminobenzidine (DAB) reagent kit (Cat. No. 00-2014; Invitrogen, Carlsbad, CA, USA). The immunostained sections were counterstained with hematoxylin. Apoptosis was detected by the terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling (TUNEL) method (Cat. No. 4810-30-K; Trevigen, Gaithersburg, MD, USA).

The masseter muscles were resected from the mandibles and embedded in paraffin. Serial sagittal sections were made at 7 μm thickness, deparaffined and H-E stained.

Statistical analysis

The distribution of the data in the samples was analyzed for skewness and kurtosis, and the parametric *t* test was used on the assumption that the samples were normally distributed. The body weights, rates of weight gained, and mandibular measurements were evaluated for the presence of inter-group differences with a one-way ANOVA. Differences between specific groups were assessed with Duncan's multiple range tests.

Experimental set 2

The mandibular measurements of the two groups of untreated rats having the same age but different body

Table 1 Mean body weight (g) of the three groups of the experimental set1 measured weekly during the experimental period and mandibular measurements at the time of sacrifice (8 weeks of age) of those groups

	Group 1	*Group 2*	*Group 3*
Weight (g)			
Week 4	124.05 (± 6.03)	124.75 (± 4.76)	124.70 (± 3.78)
Week 5	166.62 (± 7.08)	165.45 (± 7.92)	151.91 (± 13.00)[a]
Week 6	240.11 (± 9.49)	237.23 (± 7.54)	222.68 (± 13.58)[a]
Week 7	277.51 (± 11.37)	276.06 (± 10.36)	263.79 (± 13.10)[a]
Week 8	318.17 (± 13.21)	313.08 (± 13.65)	299.40 (± 16.83)[a]
Mn Measurements (mm)			
Mandibular body length	20.64 (± 0.32)	20.47 (± 0.32)	20.09 (± 0.46)[b]
Condylar length	21.88 (± 0.24)[c]	21.71 (± 0.24)[c]	21.60 (± 0.39)[c]
Coronoid process length	20.12 (± 0.35)	20.00 (± 0.30)	19.75 (± 0.36)[b]
Anterior region height	4.82 (± 0.13)	4.81 (± 0.12)	4.65 (± 0.12)[b]
Coronoid process height	13.37 (± 0.21)	13.33 (± 0.24)	12.83 (± 0.32)[b]
Condylar height	11.16 (± 0.27)	11.17 (± 0.26)	10.26 (± 0.37)[b]

Values are means (± s.d.). Number of animals in each group is 20.
Group 1: control group. Group 2: saline group. Group 3: botulinum toxin type A group.
[a]Mean is significantly different from those of group1 and group2 ($P < 0.05$).
[b]Mean is significantly different from those of group1 and group2 ($P < 0.05$).
[c]Mean is significantly different between each groups ($P < 0.05$).

weight were evaluated. Ten rats that weighed equally as the control group were assigned group A (large animal group), while the other 10 rats that weighed equally as the BTX-A group were assigned group B (small animal group).

All animals were weighed and sacrificed in the same manner as in the previous experimental design. Dry mandibles were prepared, measurements were made, and statistical analysis was doneusing the same methods previously described.

Results

Experimental set 1
Mandibular measurements
The mandibular measurements of baseline control group were normally distributed. The results of the body weight and mandibular measurements of the three groups are shown in Table 1. While there were no differences among the body weight of the three groups atthe start of the trials, the body weight of BTX-A group was less than those of Control and saline group ($P < 0.05$)in the course of the experiment. Figure 2 represents bar graphs of the mandibular measurements of the three groups. In all six mandibular measurements, the BTX-A group had areduced dimension compared with the control and saline group ($P < 0.05$). Differences between the measurements of control and saline group were not statistically significant, except for condylar length ($P < 0.05$).

Histological study
The condylar cartilages of all the experimental groups showed four major zones (Shen and Darendeliler, 2005) distinctively; the fibrous covering, reserve, proliferation and hypertrophic zones (Figure 3D). However, the proliferation and hypertrophic zones of the BTX-A group were thinner than those of the other groups (The thickness of a–d in Figure 3D, E, F were compared).

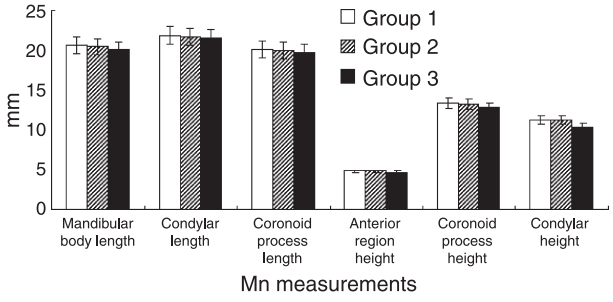

Figure 2 Mandibular measurements of the experimental set 1. Vertical bars represent s.d. Group 1: control group, Group 2: saline injection group, Group 3: botulinum toxin type A injection group

Immunoreactions for PCNA were similar in all three groups (Figures 3G–I). Immunopositive reaction was detected mainly in the cells of the proliferation zone and slightly positive reaction was shown in the cells of the reserve and hypertrophic zones. The fibrous covering layer showed a negative reaction.

In all groups, TUNEL-positive cells were observed in the proliferation- and hypertrophic zones (Figures 3J–L). Negative reaction was detected in the fibrous covering and reserve zones. A main difference was observed in the proliferation zone. Specimens of the BTX-A group showed a strong positive reaction to TUNEL stain in the proliferation zone especially in the starting area of this zone.

In cross-sections of the masseter muscle, muscle fibers of the control and saline groups had a normal polygonal shape without necrosis or inflammation, and the fiber size was uniform (Figures 4a,b,d and e). Nuclei were located at the periphery of the fibers in a subsarcolemmal position. On the other hand, diameters of the muscle fiber of the BTX-A group were more irregular and the nuclei were packed more densely than those of the control and saline group (Figures 4c and f).

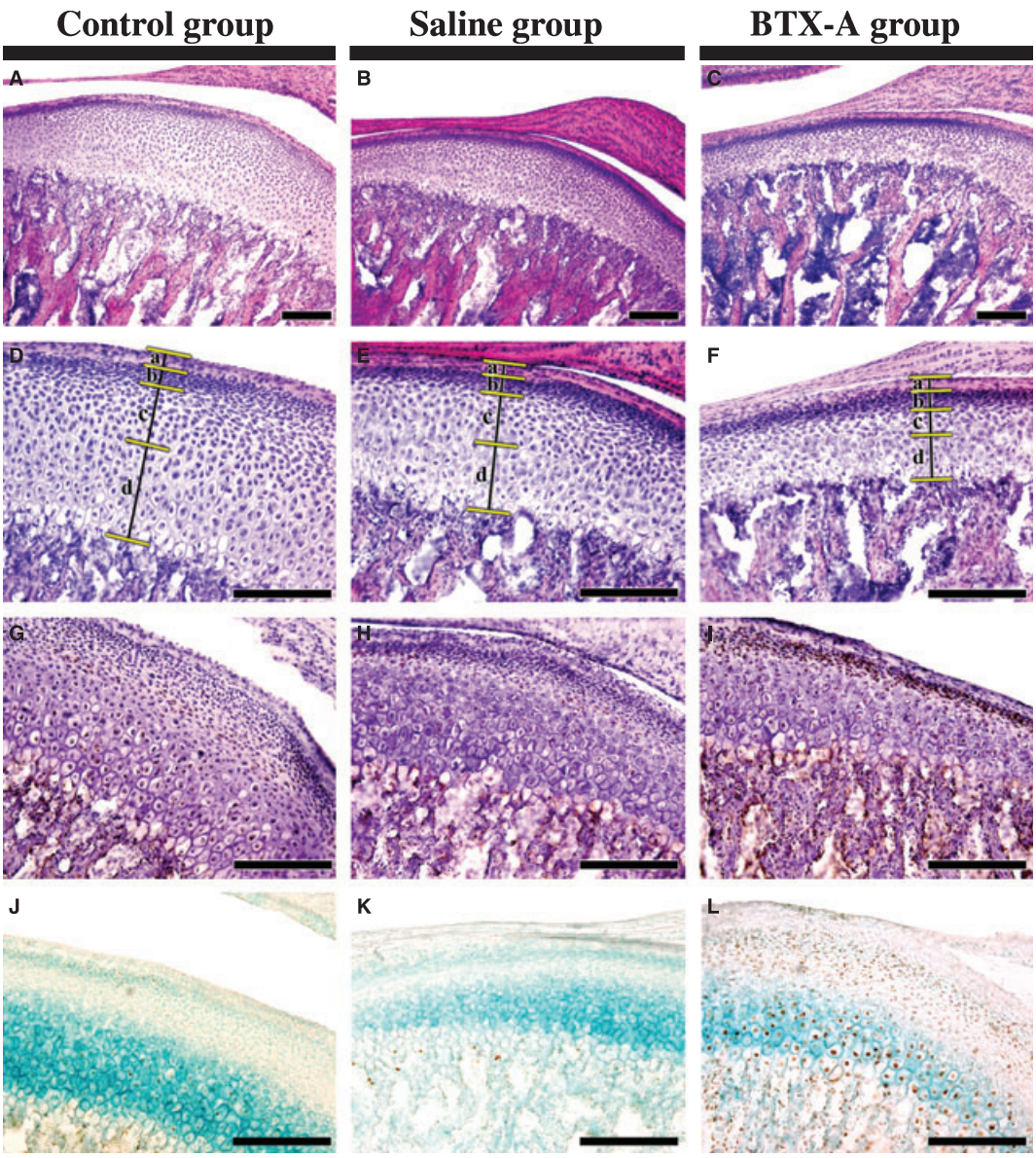

Figure 3 Histological analysis and localization patterns of proliferation cell nuclear antigen (PCNA) and terminal deoxynucleotidyl transferase mediated dUTP nick-end-labeling (TUNEL) staining. (**A–F**) The condylar cartilages of control, saline and botulinum toxin type A (BTX-A) group are stained by HE staining. (**A, D**) The condylar cartilage is shown in the control group ('**D**' is higher magnification of '**A**'). (**D**) The fibrous covering (a), reserve (b), proliferation (c) and hypertrophic (d) zones can be seen. (**B, E**) The condylar cartilage is shown in the saline group ('**E**' is higher magnification of '**B**'). (**C, F**) the proliferation and hypertrophic zones of the BTX-A group were thinner than those of the other groups ('**F**' is higher magnification of '**C**'). (**G–I**) PCNA immunohistochemistry and (**J–L**) TUNEL assay are used to define cellular modulations. (**G–I**) There are most PCNA-positive cells localized in the proliferation zone. PCNA positive reaction was similar in all three groups. (**J–L**) In all groups, TUNEL positive cells were observed in the proliferation and hypertrophic zones. (**L**) In the BTX-A group showed stronger positive reaction than other groups. (All scale bars, 200 μm)

Interestingly, in the muscle of the BTX-A group, few muscle fibers with central nuclei were observed.

Experimental set 2

The measurements of mandible for each groups are summarized in Table 2 and Figure 5. The differences between the group A and B were not statistically significant, except for the mandibular body length (Me-Go; see Figure 1). The mandibular body length was longer in the group A and shorter in the group B ($P < 0.05$).

Discussion

The mandibular condyle has been the focus of numerous studies investigating local growth-control mechanisms because of its presumed sensitivity to variations in the local mechanical environment. Local mechanical conditions affecting the condyle have been altered in various ways, including condylectomy, intermaxillary fixation, removal of incisors, alteration of dietary consistency, muscle resection, and application of orthodontic appliances (Bouvier and Hylander, 1984). The literature

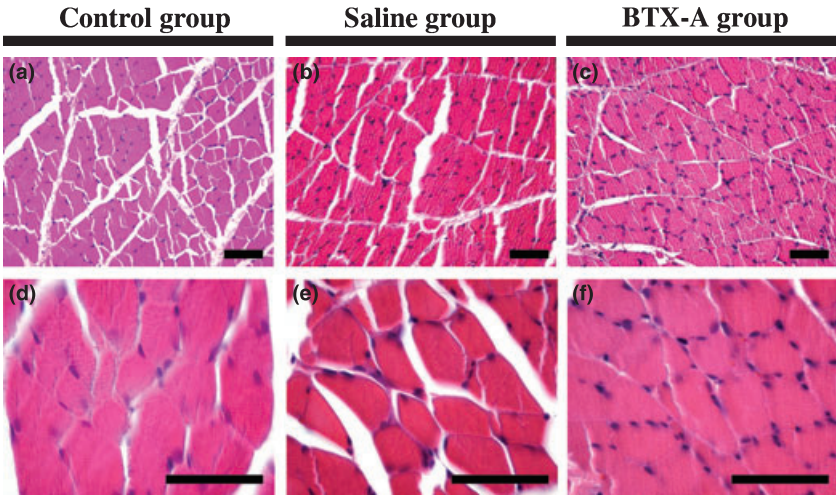

Figure 4 HE staining of control, saline and botulinum toxin type A (BTX-A) group. (**a–f**) In cross sections of the masseter muscle are examined by HE staining. (**a, b, d** and **e**) The muscle fibers of the control and saline group have a normal polygonal shape without necrosis or inflammation and the fiber size was uniform (‘**d**’ is higher magnification of ‘**a**’. ‘**e**’ is higher magnification of ‘**b**’). (**c, f**) The muscle fiber of the BTX-A group were more irregular and the nuclei were packed more densely than those of the control and saline group (‘**f**’ is higher magnification of ‘**c**’). (All scale bars, 50 μm)

Table 2 Mandibular measurements of the experimental set 2: mean (± s.d.)

Measurements (mm)	*Group A*	*Group B*
Mandibular body length	20.93 (± 0.39)	20.64 (± 0.34)*
Condylar length	21.94 (± 0.28)	21.76 (± 0.30)
Coronoid process length	20.30 (± 0.49)	20.11 (± 0.28)
Anterior region height	4.87 (± 0.16)	4.77 (± 0.12)
Coronoid process height	13.37 (± 0.27)	13.30 (± 0.23)
Condylar height	11.32 (± 0.20)	11.21 (± 0.22)

Number of animals in each group is 7.
Group A: large animal group. Group B: small animal group.
*Indicates a significant difference between the groups ($P < 0.05$).

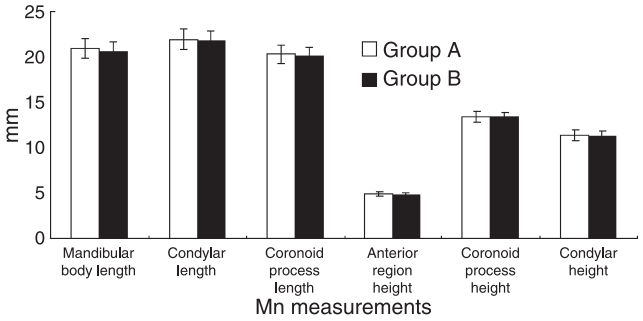

Figure 5 Mandibular measurements of the experimental set 2. Vertical bars represent s.d. Group A: large animal group; Group B: small animal group

reviews were focused on the latter three methods as this study was to investigate the effects of reduced muscle tonicity induced by BTX-A on the mandibular growth. Many experiments have shown that a low masticatory function resulted in smaller dimensions of the mandible and⁄or condyle and topographic changes in the cartilage thickness (Bouvier and Hylander, 1984; Asano, 1986; Hinton and Carlson, 1986; Mcfadden *et al*, 1986; Ghafari and Cowin, 1989; Kiliaridis, 1989; Easton and Carlson, 1990; Hinton, 1991; Yamada and Kimmel, 1991; Tuominen *et al*, 1993, 1994; Kantomaa *et al*, 1994; Monje *et al*, 1994; Navarro *et al*, 1995; Kiliaridis *et al*, 1996; Ulgen *et al*, 1997; Bouvier, 1998; Bresin *et al*, 1999; Kiliaridis *et al*, 1999; Maki *et al*, 2002). These findings are in agreement with the present study. The detailed discussions of the results of this study are as follows.

Mandibular measurements

According to the results, the mandibles of the rats in the BTX-A group had areduced dimension, compared with the control and saline groups. Although the control and saline groups showed novariations generally, condylar length showed a difference between the two groups. We assume that the result might be influenced by observational error because of the difficulty in locating the condylar point.

As the mean body weight of the BTX-A group was lower than that of the other two groups, the experimental set 2 was designed to determine whether body weight (size) influences mandibular measurements. According to the results, differences in mandibular measurements in the experimental set 1 can be considered to result from BTX-A injection. While the weight difference itself might be the result of reduced muscle tonicity induced from the BTX-A injection, one must not jump to conclusions by excluding othercauses.

Histologic study

In order to determine the causes of the difference between the BTX-A group and the other two groups, cell proliferation was evaluated using PCNA. Even though the control group had the thickest proliferation zone, strong cell proliferation in comparison with the BTX-A group was not detected. Therefore, cell proliferation could not account for the differences in the cartilage layer thickness among the groups.

In addition, TUNEL staining was performed to evaluate the effects of cell death on condylar growth. TUNEL staining was used to detect apoptosis. The proliferation zone of the BTX-A group showed increased apoptosis compared with the control and saline groups. The most severe cell death was detected in the proliferation zone adjacent to the reserve zone.

This result suggests that muscle atrophy induced by BTX-A injection did not affect the fibrous covering and reserve zone of the condylar cartilage. The fibrous covering is functionally important and the reserve zone has a growth potential (Kierszenbaum, 2002). Thus the condyle preserves growth potential and there is no functional impairment. However, it is thought that induced cell death at the proliferation stage of the reserve zone inhibits the growth of the mandible and condyle.

Regarding histologic assessment of muscle fiber, these results implicate for reflection of muscle denervation after BTX-A injection (Vilmann *et al*, 1990; Borodic and Pearce, 1994). The formation of condensed myofibers without inflammatory reaction in the BTX-A injection group might be an expression of its degenerative atrophy (Akagawa *et al*, 1983). In BTX-A group, some muscle fibers with central nuclei can be regarded as developing regenerated fibers after degeneration (Nishide *et al*, 2001).

This investigation is a preliminary study for the application of BTX-A on the masseter muscle to regulate the local mechanical environment of mandibular growth as a means of reducing muscle tonicity. In order to use BTX-A as an adjunctive treatment in orthodontic and/or orthopedic treatment for growing patients, safety of pediatric use should be proved and pediatric dose should be determined. Therefore, further investigations should be performed to find the precise minimum effective dose in animal study. In addition, in order to determine the most effective age, further investigation at the different ages should be performed. The differences in the mean body weight in this study could be a bias. To eliminate this variable, unilateral injection could be considered for further study.

Taken together, it is shown that BTX-A injection clearly has an influence on inhibitory action of the developing mandible because of apoptosis at the proliferation stage of the reserve zone. Furthermore, these studies strongly implicate that clinicians must be cautious in dealing with injecting BTX-A to children.

Acknowledgement

This study was supported by grants from HANALL Pharmaceutical Co., Seoul, Korea.

References

Akagawa Y, Nikai H, Tsuru H (1983). Histologic changes in rat masticatory muscles subsequent to experimental increase of the occlusal vertical dimension. *J Prosthet Dent* **50:** 725–732.

Asano T (1986). The effects of mandibular retractive force on the growing rat mandible. *Am J Orthod Dentofacial Orthop* **90:** 464–474.

Borodic GE, Pearce LB (1994). New concepts in botulinum toxin therapy. *Drug Saf* **11:** 145–152.

Bouvier M (1998). Effects of age on the ability of the rat temporomandibular joint to respond to changing functional demands. *J Dent Res* **67:** 1206–1212.

Bouvier M, Hylander WL (1984). The effect of dietary consistency on gross and histologic morphology in the craniofacial region of young rats. *Am J Anat* **170:** 117–126.

Bresin A, Kiliaridis S, Strid K-G (1999). Effect of masticatory function on the internal bone structure in the mandible of the growing rat. *Eur J Oral Sci* **107:** 35–44.

Carruthers A, Carruthers J (1998). Clinical indications and injection technique for the cosmetic use of botulinum A exotoxin. *Dermatol Surg* **24:** 1189–1194.

Carruthers A, Carruthers J (2001). Botulinum toxin type A: history and current cosmetic use in the upper face. *Semin Cutan Med Surg* **20:** 71–84.

Easton JW, Carlson DS (1990). Adaptation of the lateral pterygoid and superficial masster muscles to mandibular protrusion in the rat. *Am J Orthod Dentofacial Orthop* **97:** 149–158.

Freund B, Schwartz M, Symington JM (1999). The use of botulinum toxin for the treatment of temporomandibular disorders: preliminary findings. *J Oral Maxillofac Surg* **57:** 916–920.

Ghafari J, Cowin DH (1989). Condylar cartilage in the muscular dystrophic mouse. *Am J Orthod Dentofacial Orthop* **95:** 107–114.

Hinton RJ (1991). Jaw protruder muscles and condylar cartilage growth in the rat. *Am J Orthod Dentofacial Orthop* **100:** 436–442.

Hinton RJ, Carlson DS (1986). Response of the mandibular joint to loss of incisal function in the rat. *Acta Anat* **125:** 145–151.

Ivanhoe CB, Lai JM, Francisco GE (1997). Bruxism after brain injury: successful treatment with botulinum toxin-A. *Arch Phys Med Rehabil* **78:** 1272–1273.

Jankovic J, Brin MF (1997). Botulinum toxin; historical perspective and potential new medications. *Muscle Nerve* **20**(Issue S6)**:** S129–S145.

Kantomaa T, Tuominen M, Pirttiniemi P (1994). Effect of mechanical forces on chondrocyte maturation and differentiation in the mandibular condyle of the rat. *J Dent Res* **73:** 1150–1156.

Kierszenbaum AL (2002). Osteogenesis in histology and cell biology. *An introduction to pathology*, Mosby: St. Louis, pp. 133–142.

Kiliaridis S (1989). Muscle function as a determinant of mandibular growth in normal and hypocalcaemic rat. *Eur J Orthod* **11:** 298–308.

Kiliaridis S, Bresin A, Holm J, Strid K-G (1996). Effects of masticatory muscle function on bone mass in the mandible of the growing rat. *Acta Anat* **155:** 200–205.

Kiliaridis S, Thilaner B, Kjellberg H, Topouzelis N, Zafiriadis A (1999). Effect of low masticatory function on condylar growth: a morphometric study in the rat. *Am J Orthod Dentofacial Orhtop* **116:** 121–125.

Maki K, Nishioka T, Shioiri E, Takahashi T, Kimura M (2002). Effects of dietary consistency on the mandible of rats at the growth stage: computed X-ray densitometric and cephalometric analysis. *Angle Ortod* **72:** 468–475.

Mandel L, Tharakan M (1999). Treatment of unilateral masseteric hypertrophy with botulinum toxin: case report. *J Oral Maxillofac Surg* **57:** 1017–1019.

Mcfadden LR, McFadden KD, Precious DS (1986). Effect of controlled dietary consistency and cage invironment on the rat mandibular growth. *Anat Rec* **215:** 390–396.

Monje F, Delgado E, Navarro MJ, Miralles C, del Hoyo J (1994). Changes in the temporo-mandibular joint caused by the vertical facial pattern. Study on an experimental model. *J Craniomaxillofac Surg* **22:** 361–370.

Moore AP, Wood GD (1994). The medical management of masseteric hypertrophy with botulinum toxin type A. *Br J Oral Maxillofac Surg* **32:** 26–28.

Nakata S (1981). Relationship between the development and growth of cranial bones and masticatory muscles in postnatal mice. *J Dent Res* **60:** 1440–1450.

Navarro M, Delgado E, Monje F (1995). Changes in mandibular rotation after muscular resection. Experimental study in rats. *Am J Orthod Dentofacial Orthop* **108:** 367–379.

Niamtu J (1999). Aesthetic uses of botulinum toxin A. *J Oral Maxillofac Surg* **57:** 1228–1233.

Nishide N, Baba S, Hori N, Nishikawa H (2001). Histological study of rat masseter muscle following experimental occlusal alteration. *J Oral Rehabil* **28:** 294–298.

Pearce LB, Borodic GE, First ER, MacCallum RD (1994). Measurement of botulinum toxin activity: evaluation of the lethality assay. *Toxicol Appl Pharmacol* **128:** 69–77.

Scott AB, Magoon EH, McNeer KW, Stager DR (1989). Botulinum treatment of strabismus in children. *Trans Am Ophthalmol Soc* **87:** 174–180.

Shen G, Darendeliler MA (2005). The adaptive remodeling of condylar cartilage – a transition from chondrogenesis to osteogenesis. *J Dent Res* **84:** 691–699.

Smyth AG (1994). Botulinum toxin treatment of bilateral masseteric hypertrophy. *Br J Oral Maxillofac Surg* **32:** 29–33.

Tan EK, Jankovic J (2000). Treating severe bruxism with botulinum toxin. *J Am Dent Assoc* **131:** 211–216.

To E, Ahuja AT, Ho WS *et al* (2001). A prospective study of the effect of botulinum toxin A on masseteric muscle hypertrophy with ultrasonographic and electromyographic measurement. *Br J Plast Surg* **54:** 197–200.

Tuominen M, Kantomaa T, Pirttiniemi P (1993). Effect of food consistency on the shape of the articular eminence and the mandible. *Acta Odontol Scand* **51:** 65–72.

Tuominen M, Kantomaa T, Pirttiniemi P (1994). Effect of altered loading on condylar growth in the rat. *Acta Odontol Scand* **52:** 129–134.

Ulgen M, Baran S, Kaya H, Karadede I (1997). The influence of the masticatory hypofunction on the craniofacial growth and development in rats. *Am J Orthod Dentofacial Orthop* **111:** 189–198.

Vilmann H, Kirkeby S, Kronborg D (1990). Histomorphometrical analysis of the influence of soft diet on masticatory muscle development in the muscular dystrophic mouse. *Arch Oral Biol* **35:** 37–42.

Von Lindern JJ, Niederhagen B, Appel T, Berge S, Reich RH (2001). Type A botulinum toxin for the treatment of hypertrophy of the masseter and temporal muscles: an alternative treatment. *Plast Reconstr Surg* **107:** 327–332.

Yamada K, Kimmel DB (1991). The effect of dietary consistency on bone mass and turnover in the growing rat mandible. *Arch Oral Biol* **36:** 129–138.

Copyright of Oral Diseases is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.