Int. J. Oral Maxillofac. Surg. 2019; 48: 475–479
https://doi.org/10.1016/j.ijom.2018.07.003, available online at https://www.sciencedirect.com

International Journal of Oral & Maxillofacial Surgery

Clinical Paper
Oral Medicine

Botulinum toxin A treatment of epiphora secondary to autologous submandibular gland transplantation

X. F. Shan[1], L. Lv[2], Z.-G. Cai[1], G.-Y. Yu[1]
[1]Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, National Engineering Laboratory for Digital and Material Technology of Stomatology, Beijing Key Laboratory of Digital Stomatology, Beijing, China; [2]Department of Ophthalmology, Affiliated Beijing Tong Ren Hospital, Capital University of Medical Science, Beijing, China

X.F. Shan, L. Lv, Z.-G. Cai, G.-Y. Yu: Botulinum toxin A treatment of epiphora secondary to autologous submandibular gland transplantation. Int. J. Oral Maxillofac. Surg. 2019; 48: 475–479. © 2018 Published by Elsevier Ltd on behalf of International Association of Oral and Maxillofacial Surgeons.

Abstract. The aim of this study was to explore whether botulinum toxin A (BTXA) injection treats epiphora secondary to submandibular gland (SMG) transplantation for severe keratoconjunctivitis sicca.

Fifteen patients with epiphora after SMG transplantation were separated to three groups, and received 15 U, 20 U and 25 U BTXA injection in the transplanted SMG, respectively. Secretion of transplanted SMG was assessed subjectively by visual analogue scale (VAS) regarding epiphora, and objectively by Schirmer test.

There were no significant differences in the 15-U BTXA group regarding the values of the VAS on epihora before and 1 month after BTXA injection. While in 20-U group and 25-U group, the values of VAS on epihora decreased significantly after BTXA injection, and lasted for 6 months. Under resting conditions, the secretion of transplanted SMG decreased 64.4%, 73.0% and 78.0% in 15-U, 20-U and 25-U groups, respectively ($P < 0.01$), in 1 month after BTXA injection; significant secretion decreasing lasted 3 months only in the 25-U BTXA group.

BTXA injection can decrease the secretion of transplanted SMG significantly, relieving the symptoms of epiphora; 25 U BTXA is a suitable dose to treat 'opportunistic epiphora' after SMG transplantation.

Key words: botulinum toxin A; submandibular gland; epiphora.

Accepted for publication
Available online 20 July 2018

Keratoconjunctivitis sicca (KCS), or dry-eye syndrome, is a relatively common disease. In cases of absolute dry eye, severe pain and blindness may occur due to ocular surface failure. With severely dry eye, affected individuals can also suffer significant pain, have a risk of sight-threatening corneal infection and ulceration, and lose their sight completely[1]. Autotransplantation of the submandibular gland (SMG) offers an effective approach for providing tear substitution and preventing damage to the cornea from KCS. Studies have shown that with successful transplantation, symptoms of dry eye disappear and discomfort from bright light is relieved[1–6]. In approximately 40% of viable grafts, however, epiphora may occur due to oversecretion of the transplanted SMG. This condition may lead to social embarrassment and blurred vision

0901-5027/040475 + 05

due to a corneal edema, and may require a secondary procedure to reduce the size of the graft[1,2,7]. For patients with relatively mild epiphora, topical application of modified atropine gel is effective[8], but is insufficient to control symptoms in more severe cases.

The cause of epiphora is believed to result from the surgical denervation of the gland, which affects the secretory mechanism of the transplanted SMG. Therefore, physical exercise or local hyperthermia can increase the secretion of transplanted SMG resulting in excessive tearing, or 'opportunistic' epiphora. Opportunistic epiphora occurs primarily during the period from May to October when air temperature is relatively high, but is rare from November to April due to the relatively low air temperature[8]. Therefore, a reversible therapy seems like an ideal form of treatment for the condition.

Botulinum toxin type A (BTXA) is a neurotoxin that inhibits presynaptic acetylcholine release from nerve endings, thereby interfering with nerve impulses. After BTXA-induced paralysis, the baseline functioning of the neuromuscular junction gradually recovers over a period of months. Local effects of BTXA, without systemic effects, have led rapidly to its application in various neuromuscular and movement disorders, such as dystonia and spasticity[9,10]. BTXA has also been used to treat excessive glandular secretion (e.g., hyperhidrosis, hyperlacrimation, allergic rhinitis and excessive drooling) with encouraging results[11–13]. In 2002, Keegan et al. reported a case of epiphora secondary to autologous SMG transplantation for KCS that was treated with BTXA. Treatment produced a marked decrease in secretions which lasted for 3 months. This case report highlighted the need for further research with a larger cohort to investigate the efficacy of BTXA for epiphora in this particular groups of patients[14].

Considering that opportunistic epiphora occurs for a period of 3–5 months every year, it would be convenient if the duration of effect for BTXA was more than 3 months. This study was designed to investigate the efficacy, duration of activity, appropriate dosage, and possible side effects of BTXA injection for opportunistic epiphora secondary to SMG transplantation in former KCS patients.

Patients and methods

Patients

This study was approved by the Ethics Committee for Human Experiments at Peking University School and Hospital of Stomatology (PKUSSIRB-201413040). All possible adverse effects and risks of the study were explained to all of the patients, and written informed consent was obtained.

The indications for SMG transplantation were that the patients had persistently pronounced symptoms of dry eye, and that other previous ophthalmologic treatments had failed. Ophthalmologic evaluation showed that a Schirmer test value of <2 mm, a break-up time of tear film value of <5 s, and a positive fluorescence staining of the cornea.

There were 15 patients (four males, 11 females) enrolled in the study between June 2009 and November 2014, and all had opportunistic epiphora secondary to microvascular SMG transplantation for severe KCS. Steven–Johnson's syndrome (six cases) was the main aetiology of KCS in this series, followed by acute conjunctivitis (three cases) and other aetiologies (three cases). The aetiology of three cases was unclear. The mean age of the patients was 38 years (range, 20–73 years). Eight patients had previously had reduction surgery of the transplanted SMG to decrease glandular secretion. Among them, six patients had undergone a reduction procedure once; and two patients had undergone a reduction procedure twice. Inclusion and exclusion criteria are described in Table 1 (note, one patient had received a partial SMG transplantation, and the rest had entire gland transplants). Before treatment, patients completed a VAS for epiphora. Each patient was asked to score the severity and frequency of tearing (from 0 to 10). The required score on the VAS, representing the degree of epiphora, for enrollment in the study was greater than six.

Design of the study

Outcome measurement was divided into subjective and objective assessments. In the subjective assessment portion, VAS on epiphora and its impact on the patients were assessed at baseline, and at 1, 2, 3, 4, 5 and 6 months after BTXA injection. The patients completed VAS at every assessment. A decrease of two or more grades from baseline in the VAS score was defined as an effective treatment.

The objective assessment included a clinical evaluation that consisted of visual acuity testing and slit-lamp microscopy. It also included Schirmer test 1 to measure secretion of the transplanted SMG as we previously reported[15]. Briefly, without topical anaesthesia, the folded end of Whatman no. 41 paper (5×120 mm) was inserted into the lateral side of the lower conjunctival fornix. The Schirmer test was performed in a quiet location at a room temperature of 23 °C. The test was conducted in two conditions: basal (or resting) and with physical activity. The former was to represent unstimulated secretion; the latter, stimulated secretion. In the basal condition, the patients rested for 30 min with no physical activity or glandular stimulation, and the Schirmer test was conducted the first time. Then, after physical activity (climbing six flights of stairs in 3 min), the Schirmer test was immediately repeated. The results were recorded for both the unstimulated (first test) and stimulated (second test) conditions.

Examination by slit-lamp microscopy was performed to check for the occurrence of eyelid entropion, corneal opacities or corneal neovascularization after BTXA injection. Examinations of visual acuity, and slit-lamp microscopy were measured at baseline and again 1 month after BTXA injection. Schirmer test was conducted at baseline, 1 month, 3 months and 6 months after BTXA injection.

Fifteen patients were divided into three groups according to the dosage of BTXA injection. Each group consisted of five

Table 1. Inclusion and exclusion criteria.

Inclusion criteria
- >3 months since submandibular gland transplantation for keratoconjunctivitis sicca
- Severe epiphora primarily in during the summer
- Visual analogue scale for epiphora greater than six
- Schirmer test result >30mm/5min
- Age >20 years
- Informed consent given

Exclusion criteria
- Treatment with botulinum toxin A before
- Known hypersensitivity to botulinum toxin or any part of the formulation
- Taking, or have taken, drugs that affect saliva secretion in the past 6 months
- Known systemic diseases (e.g., bronchial asthma, congenital heart failure, myasthenia gravis)
- Severe epiphora occurring year-round or in the winter
- Obstructed duct of transplanted submandibular gland

patients, and received 15 U, 20 U and 25 U BTXA injection, respectively. The patients received BTXA injection in the transplanted SMG once. The start time of treatment was in May or June.

Treatment procedures

For the injections, BTXA was diluted using 2 ml of normal saline (0.9%) per 100 U, for a concentration of BTXA solution of 5U/0.1 ml. Telephone follow-up calls were conducted every month for 6 months following BTXA injection. The patients were also asked to record side effects in a diary.

The extent of transplanted SMG can be roughly evaluated by palpation. BTXA was administered via percutaneous injections into the transplanted SMG in the temporal region (depth of injections, 1–1.5 cm; 1-ml syringe, 27-gauge needle). According to the patient group, a corresponding dose of BTXA was injected into the transplanted SMG with three separated entry points (Fig. 1). In order to avoid damage to Wharton's duct, the direction of injection was from posterior to anterior. Also, an attempt was made to aspirate after the needle was inserted to make certain BTXA was not injected directly into a blood vessel or the SMG duct.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed with SPSS for Windows, version 11.5 (SPSS, Chicago, IL, USA). Differences between the groups were analyzed using one-way analysis of variance followed by Fisher's least-significant difference post hoc tests. $P < 0.05$ was considered statistically significant.

Results

All of the patients reported that the symptoms of epiphora had greatly improved at 1 week after injection. In the 15-U group, three of five patients felt secretions from the transplanted SMG were decreased at 1 month following BTXA injection. Among the three patients, two felt the improvement from BTXA lasted for 2 months, and one for six months. Likewise, in the 20-U group, three of five patients felt that secretions from the transplanted SMG were decreased at 1 month after treatment. Among these three patients, one patient felt the duration of improvement after BTXA lasted for 1 month; one felt it lasted for 2 months, and one for six months. In the 25-U group, all of the patients reported that the transplanted SMG secretion decreased significantly after BTXA injection, and four patients felt the beneficial effect lasted for 6 months (Table 2).

There were no significant differences between mean pre- and post-treatment scores on VAS for the 15-U group; whereas in the 20-U and 25-U groups, the VAS scores decreased significantly after BTXA injection, and a significant difference persisted during the study (6 months; Fig. 2).

Table 2. Duration of effectiveness after botulinum toxin A (BTXA) injection.

Group	Cases at different durations (months) after BTXA injection						
	<1	1	2	3	4	5	6
15 U	2		2				1
20 U	2	1	1				1
25 U				1			4

Analysis of the objective Schirmer test results showed that, under rest conditions at 1 month after BTXA injection, the mean reduction in the secretion values were significant for all groups: mean secretion was decreased by 64%, 73%, and 78% for 15-U, 20-U and 25-U groups, respectively ($P < 0.01$ for all groups); the mean stimulated secretion values decreased by 40%, 52% and 84% in the respective groups at 1 month ($P < 0.01$ for all groups). By 3 months, there was a significant difference between the mean secretion measurements for the 25-U group under basal conditions (it decreased by 29.0%; $P < 0.05$). No difference was found under stimulated conditions (Fig. 3). At the 6-month point after BTXA injection, no differences were found for any of the groups.

Only two patients complained of thicker, more viscous secretions after BTXA treatment (one in the 20-U group, another in the 25-U group). This situation lasted for 2 months. There were no other adverse side effects reported by the patients. Furthermore, objective measurements of visual acuity and slit-lamp microscopy at 1 month showed no side effects on vision or the ocular surface from BTXA injection. In fact, visual acuity improved in one patient at 1 month in the 25-U group.

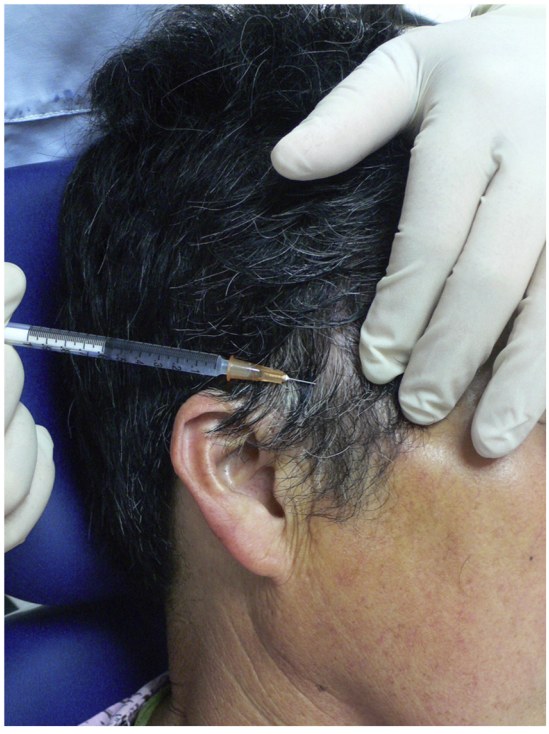

Fig. 1. Botulinum toxin A was administered via percutaneous injections guided by palpation.

Discussion

Salivary secretion is controlled by both sympathetic and parasympathetic autonomic nerves. Fluid and electrolyte secretion from salivary glands is primarily evoked by the action of acetylcholine on muscarinic acetylcholine receptors (mAChRs) and norepinephrine on α-adrenoceptors, whereas protein secretion is mainly evoked by isoproterenol on β-adrenoceptors[16,17]. Glandular fluid and ion secretion are mainly regulated by the release of acetylcholine from para-

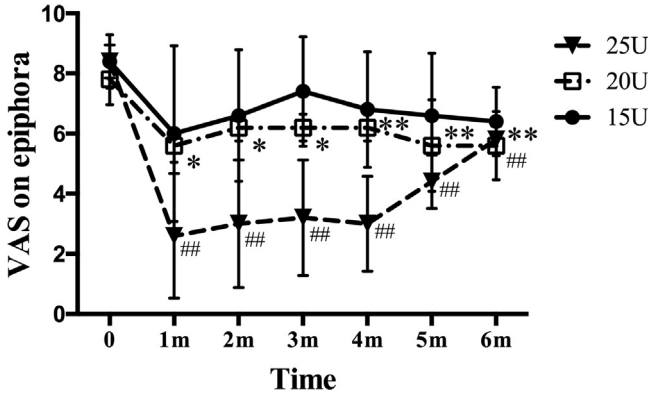

Fig. 2. Values of visual analogue scale (VAS) on epiphora changed before and 6 months after botulinum toxin A injection (20-U group: $*P < 0.05$, $**P < 0.01$ vs. control; 25-U group: $\#\#P < 0.01$ vs. control). m, months.

sympathetic nerves and its interaction with mAChR. We published a previous study showing increased mRNA and protein expression of M1- and M3-mAChR in the transplanted SMG glands of patients with epiphora[18]. In normal rabbit SMGs, inhibited redistribution of aquaporin-5 (AQP5) resulted in decreased SMG secretions[19]. Therefore, it is possible that inhibited release of Ach from the parasympathetic nerve terminal leads to decreased expression of the M3 receptor and AQP5. On this basis, we designed this clinical study to investigate the efficacy of BTXA injection in controlling epiphora secondary to SMG transplantation.

VAS was used for subjective assessments in this study. The results showed that the mean value of VAS decreased substantially and remained at significant levels for 6 months in group 3 (25-U dose), indicating BTXA injection could improve symptoms for patients with severe epiphora.

The results showed that BTXA injection could decrease the secretion of transplanted SMG significantly under both conditions of rest and physical activity in the 25-U group: secretory flow rates decreased by 78% and 84%, respectively, at 1 month after treatment with 25-U BTXA. The effectiveness of BTXA was maintained at the basal condition 3 months after treatment. These results further confirmed the effectiveness of BTXA injection in controlling severe epiphora.

In previous studies, when BTXA was used in nontransplanted SMGs to reduce excessive drooling, no conclusion was drawn as to the appropriate dosage. In published reports, the dose of BTXA has varied from 5 U/gland to 25 U/gland, for which the maximum subjective response rate was 80%[9,11,20]. In the one case that was previously reported of the treatment of epiphora after SMG transplantation by periglandular injection of Dysport, the total dose administered was 1200 U. With this dose, a subjective improvement in symptoms was achieved, although the patient received a repeat injection of 1000 U 3 months later because of increased tearing[14]. Compared to periglandular injection, BTXA can be easily spreaded to the whole transplanted SMG with a low dosage by intra-gland injection. Our study showed that the effect of BTXA was dose-dependent. Both subjective and objective assessments demonstrated significantly better results in the 25-U group compared with the other groups (15 U and 20 U). Therefore, we recommend 25 U as the appropriate dosage for controlling epiphora after SMG transplantation.

The volume of injection in studies with BTXA treatments has not been clearly described elsewhere in the literature. Jongerius et al. suggested in his study of the treatment of excessive drooling, that BTXA should be diluted in a volume of 1–1.5 ml saline before injection into SMG to achieve adequate spreading and to diminish the risk of diffusion into surrounding structures[21]. Considering the size of transplanted SMG may decrease, in our study, the volume used to mix with BTXA was 0.1–0.2 ml per injection, but the total BTXA dose was divided into three injections, each given at a different entry point (for a total of 0.3–0.6 ml) for every SMG. This method of administration was used to ensure that BTXA was distributed throughout the gland, and was less likely to diffuse into neighbouring tissues.

The reported duration of effect for BTXA treatments has varied among studies (ranging from 8 to 16 weeks)[11–13]. In our study, the time period for which there was a significant treatment effect for the 25-U group was 3 months by objective assessment, and 6 months by subjective assessment. General speaking, the months June, July, and August have the highest temperatures. Therefore, the beginning of June is a suitable time for injection of BTXA. When the function of BTXA decreased, the epiphora relieved automatically because of the lower temperatures (entering the month of September). This is why some patients felt the function of BTXA lasted for 6 months.

With BTXA injections, there is a risk that larger injected volumes can diffuse from the transplanted SMGs into the neighbouring muscle groups, causing side effects. Mild side effects such as chewing difficulties, dry mouth, dysphagia, and

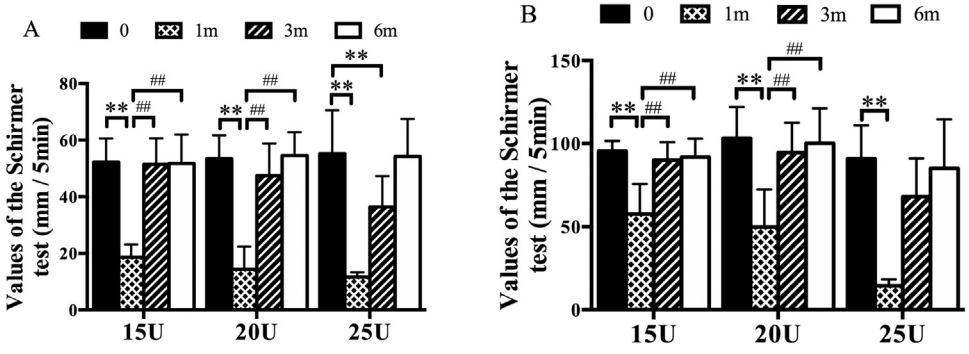

Fig. 3. (A) Schirmer testing before and 1 month, 3 months and 6 months after botulinum toxin A injection in the basal condition. (B) Schirmer testing before and 1 month, 3 months and 6 months after botulinum toxin A injection after physical activity. $**P < 0.01$ vs. control $\#\#P < 0.01$ vs. 1 m.

transient weakness of mouth closure have been reported in studies evaluating the use of BTXA for treatment of drooling[20–22]. In this study, there was less possibility of side effects from diffusion because there are only the temporalis muscle and skin around the transplanted SMG. Two patients complained of transient thicker secretions at 2 months, more viscous secretions after treatment suspected to have been caused by a temporary dramatic decrease in SMG secretions. Lastly, the question of whether the injected BTXA in transplanted SMG has any effect on the ocular surface has been addressed. No changes in the ocular surface of patients using slit-lamp exam or decreases in visual acuity were found in our series.

Different modalities for controlling epiphora secondary to SMG transplantation have their indications. Based on a series of studies, we provide a proposal for controlling epiphora secondary to SMG transplantation: (1) transplantation of partial SMG is suggested for patients with ample SMGs with normal function, in which severe postoperative epiphora would be expected; (2) conventional reduction surgery is recommended for patients with severe epiphora which is year-round; (3) BTXA injection should be used in patients who have severe epiphora primarily in the summer; and (4) topical application of modified atropine gel is suitable for patients with relatively mild epiphora before exercise.

In conclusion, BTXA injection can decrease the secretion of transplanted SMG significantly, relieving the symptoms of epiphora; 25 U BTXA is the most appropriate dose to treat opportunistic epiphora secondary to SMG transplantation.

Funding

This study was supported by grants from the National Natural Science Foundation of China (No's 30973336, 81100762 and 81371162).

Competing interests

All authors have viewed and agreed to the submission.

Ethical approval

Ethical approval was received from the Institutional Review Board of Peking University School and Hospital of Stomatology No. PKUSSIRB-201413040

Patient consent

Patient consent was obtained.

Acknowledgements. The authors are grateful to Dr. Jun Wu MMSC from the Department of Ophthalmology, Affiliated Beijing Bo Ai Hospital, Capital University of Medical Science. She carried out much of the work with regard to patient follow-ups and ophthalomological examinations.

References

1. Sieg P, Geerling G, Kosmehl H, Lauer I, Warnecke K, von Domarus H. Microvascular submandibular gland transfer for severe cases of keratoconjunctivitis sicca. *Plast Reconstr Surg* 2000;**106**:554–60.
2. Yu GY, Zhu ZH, Mao C, Cai ZG, Zou LH, Lu L, Zhang L, Peng X, Li N, Huang Z. Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca. *Int J Oral Maxillofac Surg* 2004;**33**:235–9.
3. Geerling G, Sieg P, Bastian GO, Laqua H. Transplantation of the autologous submandibular gland for most severe cases of keratoconjunctivitis sicca. *Ophthalmology* 1998;**105**:327–35.
4. Yu GY, Wu LL, Liu XJ. Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca: a 10-year experience. *Chin J Dent Res* 2009;**12**:79–82.
5. Jacobsen HC, Hakim SG, Lauer I, Dendorfer A, Wedel T, Sieg P. Long-term results of autologous submandibular gland transfer for the surgical treatment of severe keratoconjunctivitis sicca. *J Craniomaxillofac Surg* 2008;**36**:227–33.
6. Borrelli M, Schroder C, Dart JK, Collin JR, Sieg P, Cree IA, Matheson MA, Tiffany JM, Proctor G, van Best J, Hyde N, Geerling G. Long-term follow-up after submandibular gland transplantation in severe dry eyes secondary to cicatrizing conjunctivitis. *Am J Ophthalmol* 2010;**150**:894–904.
7. Qin J, Zhang L, Cai ZG, Mao C, Liu XJ, Lv L, Zou LH, Peng X, Su JZ, Wu J, Yu GY. Microvascular autologous transplantation of partial submandibular gland for severe keratoconjunctivitis sicca. *Br J Ophthalomol* 2013;**97**:1123–8.
8. Cai JR, Shan XF, Cai ZG, Zhang X, Yu GY. A new treatment for epiphora secondary to submandibular gland transplantation: transcutaneous atropine gel. *Ocul Surf* 2014;**12**:221–6.
9. Quagliato EM, Carelli EF, Viana MA. A prospective, randomized, double-blind study comparing the efficacy and safety of type a botulinum toxins botox and prosigne in the treatment of cervical dystonia. *Clin Neuropharmacol* 2010;**33**:22–6.
10. Mezali T, Kaji R, Brin MF, Hirota-Katayama M, Kubori T, Shimizu T, Kimura J. Combined use of type A and F botulinum toxins for blepharospasm: a double-blind controlled trial. *Mov Disord* 1999;**14**:1017–20.
11. Hassin-Baer S, Scheuer E, Buchman AS, Jacobson I, Ben-Zeev B. Botulinum toxin injections for children with excessive drooling. *J Child Neurol* 2005;**20**:120–3.
12. Jongerius PH, van den Hoogen FJ, van Limbeek J, Gabreëls FJ, van Hulst K, Rotteveel JJ. Effect of botulinum toxin in the treatment of drooling: a controlled clinical trial. *Pediatrics* 2004;**114**:620–7.
13. Lin YC, Shieh JY, Cheng ML, Yang PY. Botulinum toxin type A for control of drooling in Asian patients with cerebral palsy. *Neurology* 2008;**70**:316–20.
14. Keegan DJ, Geerling G, Lee JP, Blake G, Collin JR, Plant GT. Botulinum toxin treatment for hyperlacrimation secondary to aberrant regenerated seventh nerve palsy or salivary gland transplantation. *Br J Ophthalmol* 2002;**86**:43–6.
15. Su JZ, Liu XJ, Zhang L, Yu GY. Schirmer test in transplanted submandibular gland: influencing factors and a modified measurement method. *Cornea* 2013;**32**:419–22.
16. Melvin JE, Yule D, Shuttleworth T, Begenisich T. Regulation of fluid and electrolyte secretion in salivary gland acinar cells. *Annu Rev Physiol* 2005;**67**:445–69.
17. Baum BJ, Wellner RB. *Neural Mechanisms of Salivary Gland Secretion*. Basel: Karger; 1999.
18. Ding C, Cong X, Zhang Y, Yang NY, Li SL, Wu LL, Yu GY. Hypersensitive mAChRs are involved in the epiphora of transplanted glands. *J Dent Res* 2014;**93**:306–12.
19. Shan XF, Xu H, Cai ZG, Wu LL, Yu GY. Botulinum toxin A inhibits salivary secretion of rabbit submandibular gland. *Int J Oral Sci* 2013;**5**:217–23.
20. Banerjee KJ, Glasson C, O'Flaherty SJ. Parotid and submandibular botulinum toxin A injections for sialorrhoea in children with cerebral palsy. *Dev Med Child Neurol* 2006;**28**:883–7.
21. Jongerius PH, Joosten F, Hoogen FJ, Gabreels FJ, Rotteveel JJ. The treatment of drooling by ultrasound guided intraglandular injections of botulinum toxin type A into the salivary glands. *Laryngoscope* 2003;**113**:107–11.
22. Reid SM, Johostone BR, Westbury C, Rawicki B, Reddihough DS. Randomized trial of botulinum toxin injections into the salivary glands to reduce drooling in children with neurological disorders. *Dev Med Child Neurol* 2008;**50**:123–8.

Address:
Zhi-Gang Cai
Department of Oral and Maxillofacial Surgery
Peking University School and Hospital of Stomatology
No. 22 South Avenue Zhongguancun
Haidian District
Beijing 100081
China
Tel.: +86 13 910733943;
Fax: +86 10 62173402
E-mail: czg4209@126.com