Effects of Capsaicin and Carbachol on Secretion From Transplanted Submandibular Glands and Prevention of Duct Obstruction

Jia-Zeng Su, DDS, Xiao-Jing Liu, MD,* Yang Wang, DDS,* Zhi-Gang Cai, DDS,* Lei Zhang, DDS,* Lan Lv, MD,† Zhen Wang, DDS,* Xia Hong, DDS,* and Guang-Yan Yu, DDS, PhD**

Purpose: To investigate whether capsaicin and carbachol promote secretion from and prevent duct obstruction in transplanted submandibular glands (SMGs).

Methods: This retrospective cohort study included consecutive patients with severe keratoconjunctivitis sicca and successful SMG transplantation. Patients were divided into 2 groups: an exposed group receiving both capsaicin and carbachol after surgery and an unexposed group receiving neither. Secretion changes in response to capsaicin and carbachol administration were recorded in the exposed group. The main outcome measures were the secretory flow rate and duct obstruction rate in the transplanted SMGs.

Results: Forty-four patients (44 eyes) in the unexposed group and 115 patients (128 eyes) in the exposed group were followed up for more than 3 months postoperatively. The baseline characteristics were similar between the groups. The secretory flow rate before and 5, 25, 55 minutes after administration was 1 mm (0–2 mm) (median with interquartile range), 3 mm (1–5 mm), 4 mm (2–5 mm), 1 mm (0–2.5 mm), respectively, for capsaicin and 1 mm (0–3 mm), 1050 mm (450–1500 mm), 375 mm (150–600 mm), 0 mm (0–150 mm), respectively, for carbachol ($P < 0.001$ for both). In the exposed group, 6.2% of eyes had duct obstruction, whereas 18.2% of eyes in the unexposed group had duct obstruction ($P = 0.031$) (odds ratio = 0.3, 95% confidence interval, 0.105–0.856).

Conclusions: This study provides evidence that capsaicin and carbachol effectively promote secretion from and prevent duct obstruction in transplanted SMGs during at least 3 months after transplantation.

Key Words: capsaicin, carbachol, dry eye, submandibular gland transplantation, duct obstruction

(*Cornea* 2016;35:494–500)

Severe cases of keratoconjunctivitis sicca (KCS), which is a relatively common disease, can lead to vision-threatening complications.[1] Current treatment options for KCS include artificial tear substitutes, cyclosporine eye drops, and occlusion of tear drainage,[2,3] but none of these are effective in severe cases.[4] Microvascular submandibular gland (SMG) transplantation with implantation of Wharton ducts into the upper conjunctival fornix enables permanent autologous substitution of tears by secretions from the transplanted and revascularized SMG. This procedure has been proved to be effective in patients with severe KCS. The symptoms of dry eye are reportedly relieved or disappear in patients who have successfully undergone transplantation (viable gland and unobstructed duct), including discomfort resulting from bright light and wind. Thus, these patients can stop using artificial tears. Furthermore, visual acuity also improves in some patients, and reduced staining spots and intensity on fluorescent staining are seen in postoperative ophthalmologic examinations.[4–11]

However, the transplanted gland is completely disconnected from normal nerve supply during surgery. As a result, the gland is hyposecretory for 3 months after transplantation, a period known as the "latent period."[4,9,11] During this latent period, transplanted SMGs are at a high risk of duct obstruction, a complication that results in insufficient ocular lubrication of the treated eyes and may even lead to treatment failure.[9,12,13] Therefore, effective methods to improve secretion from and prevent duct obstruction in transplanted SMGs are urgently required.[13]

Secretions from normal SMGs are primarily evoked by the action of acetylcholine on muscarinic receptors and adrenergic agonists on adrenoceptors.[14] Our previous study confirmed the functional expression of transient receptor potential vanilloid subtype 1 (TRPV1) in human SMGs and found that it was involved in regulating salivary secretion.[15]

Received for publication May 28, 2015; revision received November 28, 2015; accepted December 2, 2015. Published online ahead of print January 25, 2016.

From the *Department of Oral and Maxillofacial Surgery, Peking University School and Hospital of Stomatology, Beijing, China; and †Department of Ophthalmology, Affiliated Beijing Tong Ren Hospital, Capital University of Medical Science, Beijing, China.

The authors have no conflicts of interest to disclose.

Supported by grants from the National Natural Science Foundation of China (Nos. 81470756 and 81271161), the Ministry of Education (No. 20120001110045), and Beijing Natural Science Foundation (No. 7132201). The funding organization had no role in the design or conduct of this research.

Reprints: Guang-Yan Yu, DDS, PhD, Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology, 22 Zhong Guan Cun South St, Beijing 100081, China (e-mail: gyyu@263.net).

In rabbit models of experimental transplanted SMGs, carbachol (an agonist of muscarinic receptors) and capsaicin (an agonist of TRPV1) were found to moderate structural injury and improve the secretory function of transplanted SMGs in the early stage after transplantation.[16,17] On the basis of the principle of translational medicine, we investigated clinical application of carbachol and capsaicin to ameliorate salivary hyposecretion and prevent duct obstruction in transplanted SMGs.

MATERIALS AND METHODS

This was a retrospective comparative cohort study. The procedures used to treat the patients and examine their medical records were approved by the Ethics Committee for Human Experiments of Peking University Health Science Center (No. IRB00001052-08048) before the study began, and the study was conducted in accordance with the Declaration of Helsinki guidelines for human research. All patients provided informed consent before participation after they were explained the nature and possible consequences of the study.

Patients

Consecutive patients with severe KCS who had successfully undergone microvascular autologous SMG transplantation between August 1999 and October 2012 were enrolled. All transplantations were performed at Peking University School of Stomatology using the same technique[9] and by the same surgeons. Between July 2003 and October 2012, capsaicin and carbachol were administered to all operated patients, who comprised the exposed group. The exposed group was examined for secretory flow rate changes in response to capsaicin and carbachol. Patients who were not administered either agent between August 1999 and July 2003 were enrolled as the unexposed, control group. The inclusion criterion was a viable transplanted SMG as confirmed by ^{99m}Tc scintigraphy.[18]

Surgical Procedures

SMG autotransplantation was performed as described previously.[9] In brief, the SMG, including the facial artery, facial vein, Wharton duct, and the mucosal cuff around the ductal orifice, was harvested from the submandibular triangle in the mouth and then transferred to the temporal region. The Wharton duct was passed through a subcutaneously prepared tunnel to the upper lateral conjunctival fornix. The mucosal cuff around the orifice of the Wharton duct was sutured with the palpebral conjunctiva to form an opening in the upper lateral conjunctival fold. In some cases in which the orifices of the left and right Wharton ducts were very close to each other, harvesting of the mucosal cuff was abandoned to avoid injury to the contralateral orifice. In these circumstances, the Wharton duct had a naked end, and the ductal wall was directly sutured to the palpebral conjunctiva. For these patients, an intubated polyethylene tube was left in the Wharton duct for over 1 month.

Administration of Capsaicin and Carbachol

Zostrix cream containing 0.075% capsaicin was purchased from Medicis Pharmaceutical Corp (Phoenix, AZ). About 0.5 g Zostrix cream was externally applied to a 2- × 2-cm^2 region of the skin covering the transplanted SMGs (4–6 times a day from postoperative day 7 onward, for 3 months). Carbachol was purchased from Freda Biotechnology Corp (Jinan, Shandong, China). Adult patients received subcutaneous injections of 0.2 mg carbachol to the abdomen wall near the navel, whereas children (<16 years old) received 0.1 mg. Carbachol was administered twice during the first 3 postoperative months, once on the postoperative day 10, and then around 1 month postoperatively.

Measurement of Secretory Flow Rate

The secretory flow rate of the transplanted SMGs was measured using a modified Schirmer test,[19] between 8:00 and 10:00 AM, to eliminate possible influence of the circadian rhythm. In brief, the length of the filter paper (120 × 5 mm) moistened by secretions was recorded for 5 minutes with the patients in the resting state. The Schirmer test in this format was not suitable to test the flow rate after carbachol administration, because of the high volume of salivary flow. Therefore, the secretions were first collected (also for 5 minutes) using a capillary tube from the inferior marginal tear meniscus of the treated eye after carbachol administration and then dropped slowly onto filter paper strips (120 × 5 mm) (Whatman, Maidstone, United Kingdom), which were changed when they became completely wet. The total length of the moistened filter paper strips was considered to reflect the secretory flow rate in the Schirmer test. All these procedures were identical at all time points throughout the measurement procedure.

Data Collection

Baseline observations were age, sex, KCS etiology, case history, side affected, Schirmer test results of the treated eye before surgery, and surgery duration. The characteristics recorded for the transplanted SMGs were gland weight, Wharton duct with naked end (no mucosal cuff; included Wharton ducts intubated for over 1 month), and secretory function (secretory flow rate on postoperative day 7). Patients were followed up for at least 3 months. All patients were routinely followed up on postoperative days 9 and 10, around 1 month postoperatively, and the end of the third postoperative month. Furthermore, patients who complained of any clinical characteristics of duct obstruction were nonroutinely followed up. Follow-up data were secretory flow rates 5 minutes before administration and 5, 25, and 55 minutes after capsaicin or carbachol administration and duct obstruction in the transplanted SMGs.

To eliminate the possibility of drug interactions, capsaicin was administered 1 day before carbachol administration. The secretory flow rate was then measured on postoperative days 9 and 10 (capsaicin or carbachol was the only drug administered to the patients at this time).

Any vital transplanted SMG (confirmed by ^{99m}Tc scintigraphy) showing viscous secretions and recurrent swelling was suspected to have duct obstruction. If the gland showed no improvement in secretion after stimulations (massage, hot compress, or exercise) and had a Schirmer test value <3 mm or showed irregular main duct dilation on sialography, duct obstruction was diagnosed.[13] Duct obstruction was assessed at both routine and nonroutine follow-up for patients in both exposed and unexposed groups. The fluorescence intensity score was recorded using a standardized grading system of 0 to 3 for each of the 4 quadrants (nasal, temporal, upper, and lower) on each cornea.[20]

Statistical Analysis

Proportions were used for categorical data. Mean ± SD or median (interquartile range) was used for continuous data. To evaluate the comparability of the groups at baseline, the associations between continuous and categorical variables were analyzed using the Student *t* test (symmetric distribution) or Mann–Whitney test (asymmetric distribution) and χ^2 test, respectively. Changes in Schirmer test values in response to capsaicin or carbachol were assessed using the Friedman test (nonparametric). Then, the ranks of the Schirmer test values in the Friedman test were compared using repeated-measures analysis of variance. Differences in duct obstruction rates between the groups were evaluated using the χ^2 test. The odds ratio was calculated with a 95% confidence interval. All analyses were conducted using SPSS 13.0 (SPSS Inc, Chicago, IL), and $P < 0.05$ (2-tailed) was considered statistically significant.

TABLE 1. Baseline Patient Characteristics and Characteristics of the Transplanted SMGs

	Exposed Group (n = 128)	Unexposed Group (n = 44)	*P*
Sex, male (%)	60 (46.9)	20 (45.5)	1
Age, yrs ±SD	31.9 ± 14.2	35.5 ± 16.2	0.165
Etiology, n (%)			0.162
Steven–Johnson syndrome	76 (59.4)	19 (43.2)	
Acute conjunctivitis*	17 (13.3)	11 (25.0)	
Others	5 (3.9)	1 (2.3)	
Unknown	30 (23.4)	13 (29.5)	
Case history, yrs (IQR)	10 (1.5–18)	10 (3–15)	0.967
Schirmer test value before surgery, mm (IQR)	0 (0–0)	0 (0–0)	0.273
Side, right (%)	61 (47.7)	16 (36.4)	0.221
Surgery duration, h (IQR)	4.5 (4.5–5)	4.6 (4–5.5)	0.924
Weight of gland, g ±SD	11.3 ± 3.8	12.7 ± 3.0	0.198
No mucosal cuff† (%)	8 (6.3)	3 (6.8)	1
Schirmer test value at postoperative day 7, mm (IQR)	1.0 (0–3)	1.0 (0–1.75)	0.384

Categorical variables are described as n (%) and compared using the χ^2 test. Continuous variables with asymmetric distribution are described as median (IQR) and compared using the Mann–Whitney test. Continuous variables with symmetric distribution are described as mean (±SD) and compared using the Student *t* test.

*Patients who only complained of severe redness and irritation of the eyes as the initial symptoms were suspected to have acute conjunctivitis.

†Wharton duct had a naked end, and the ductal wall was directly sutured to the palpebral conjunctiva.

IQR, interquartile range.

RESULTS

In total, 174 eyes of 161 consecutive patients (13 patients underwent bilateral transplantation) with severe KCS who had undergone successful SMG transplantation were included in the study. Because 2 patients were lost to follow-up in the first 3 postoperative months, data collection (except secretory flow rate changes in response to capsaicin or carbachol administration) was completed for 159 patients (80 males; median age, 31 years; range, 7–71 years) and 172 eyes (81 right eyes). Ultimately, 44 patients (44 eyes) formed the unexposed group and 115 patients (128 eyes) met the criteria for inclusion in the exposed group. Complete records of the secretory flow rates 5 minutes before administration and 5, 25, and 55 minutes after capsaicin or carbachol administration were collected for 43 consecutive patients from the exposed group between April 2008 and October 2012. The records of these patients before April 2008 were incomplete and therefore not included.

Table 1 summarizes the baseline characteristics of all patients (sex, age, KCS etiology, case history, affected side, Schirmer test results for the treated eye before surgery, surgery duration) and the characteristics of the transplanted SMGs (gland weight, Wharton duct with a naked end, and Schirmer test values on postoperative day 7). No significant differences were found between the exposed and unexposed groups in either baseline or transplanted SMG characteristics.

The secretory flow rate of the transplanted SMGs showed significant changes after capsaicin or carbachol administration ($P < 0.001$ for both, Friedman test). Five minutes after capsaicin application, the median Schirmer test value increased to 3 mm (1–5 mm) from 1 mm (0–2 mm) before application. The maximum Schirmer test value of 4 mm (2–5.5 mm), indicating the peak capsaicin effect, was noted 25 minutes after application. At 55 minutes after application, the effects of capsaicin declined, and the Schirmer test value was only 1 mm (0–3 mm) (Fig. 1). Carbachol had a similar effect, except that Schirmer test values after administration were considerably higher. At the peak of the carbachol effect, the transplanted SMG produced 1050 mm (450–1500 mm) saliva in 5 minutes (Fig. 1). In fact, the salivary flow rate was strong enough to wash out the large amount of viscous deposition in the duct (Fig. 2). For patients with bilateral dry eyes, no increase in the Schirmer test value was found in the untreated eyes.

Follow-up examinations at 3 months after transplantation revealed that 6.2% (8/128) and 18.2% (8/44) of the transplanted SMGs in the exposed and unexposed groups, respectively, showed duct obstruction. Postoperative intervention with capsaicin and carbachol significantly decreased the incidence of duct obstruction ($P = 0.031$, Fisher exact test, 2-sided). The odds ratio of duct obstruction in the exposed group was 0.3 (95% confidence interval, 0.105–0.856).

Side effects included mild burning sensation of the skin after capsaicin application and mild sweating, hypersalivation,

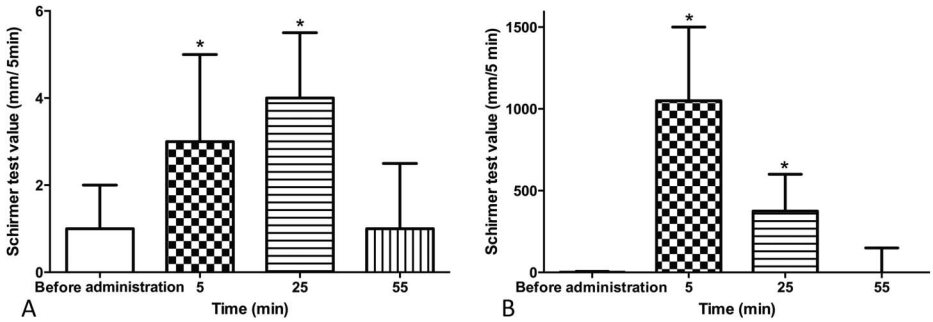

FIGURE 1. Changes in the secretory flow rate of the transplanted SMGs in response to capsaicin and carbachol on postoperative days 9 and 10, respectively (data are shown as the median with the interquartile range). A, Capsaicin. B, Carbachol. Flow rate–enhancing effects of these agents persisted for over 30 minutes after administration. Capsaicin mildly increased the secretion to a maximum of 4 mm (25 minutes after application), whereas carbachol greatly increased the secretion to 1050 mm at 5 minutes after administration. $*P < 0.05$, repeated-measures analysis of variance, compared with before administration.

increased gastric movement, and nausea after carbachol injection. In addition, mild headache and flustering were reported in 5 and 6 patients shortly after carbachol injection, respectively. No serious side effects occurred in any patients, and all these side effects spontaneously disappeared shortly after capsaicin or carbachol use.

In patients with unobstructed Wharton ducts, the secretory function of the viable transplanted SMGs remained stable. The lubricant effect of saliva effectively alleviated the dry eye symptoms. The patients could reduce and eventually stop using artificial tears (Fig. 3). The ocular surface structures also improved, as previously reported,[5,9,11] as did subjective symptoms and Schirmer test values, visual acuity, breakup time of tear film, and fluorescence staining score (Fig. 4). In patients with duct obstruction, the passageway of saliva from the transplanted glands to the eyes was blocked. The ocular surface remained dry or showed a slight sticky secretion, and the duct was dilated in some cases, referred to as obstructive sialadenitis of transplanted SMGs[13] (Fig. 5). Furthermore, the dry eye symptoms were not relieved. All 16 patients with duct obstruction received clinical interventions, but these failed and the transplanted glands lost secretory function in 5 patients, whereas other patients showed no duct obstructions after successful treatments.[13] Two of the patients who were obstruction-free at 3 months postoperatively showed continuously decreasing viscous secretions with recurrent gland swelling at 8 and 12 years postoperatively, respectively. In both cases, however, secretory function recovered after external irrigation with normal saline.[13]

DISCUSSION

Duct obstruction in transplanted SMGs seriously hampers treatment effects and can even result in failure of surgery. One of the main causes of this complication is hyposecretion from the transplanted SMG, especially during the first 3 months after transplantation or the "latent period."[4,9] Therefore, we explored new strategies to promote secretion from transplanted SMGs in the "latent period" to prevent duct obstruction.

Transplanted SMGs are disconnected from normal nerve supply, and therefore lose gustatory–salivary reflex secretion. Geerling et al[21] have found evidence regarding surviving parasympathetic ganglion cells in human transplanted SMGs, and they believe that heterologous synaptic contacts can be formed by new adrenergic axons sprouting from existing parasympathetic trunks, and that with time, these contacts can develop functional connections with parasympathetic ganglionic cells. However, this functional reinnervation cannot be expected in the short term after transplantation. Therefore, more direct stimulation using a saliva secretion-related receptor agonist has become necessary to promote secretion from transplanted glands.

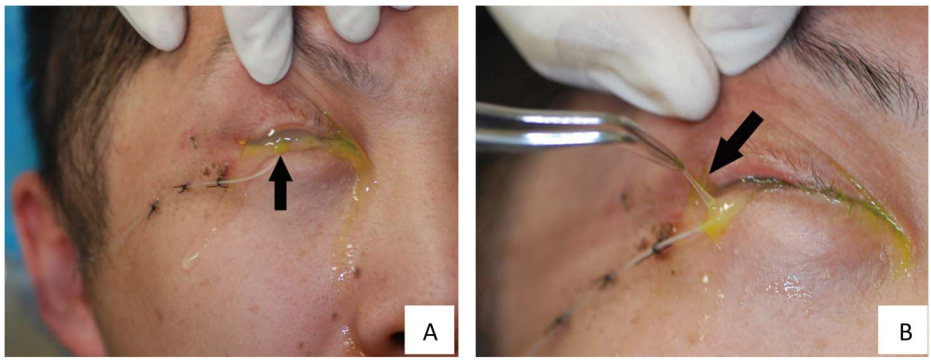

FIGURE 2. "Internal irrigation" effect of carbachol administration. A, A large amount of saliva was produced and secreted by the transplanted SMG in a relatively short time, mimicking the "internal irrigation" effect. B, This saliva washed away viscous depositions (arrow) out of the duct, along with the mucous plug (arrow).

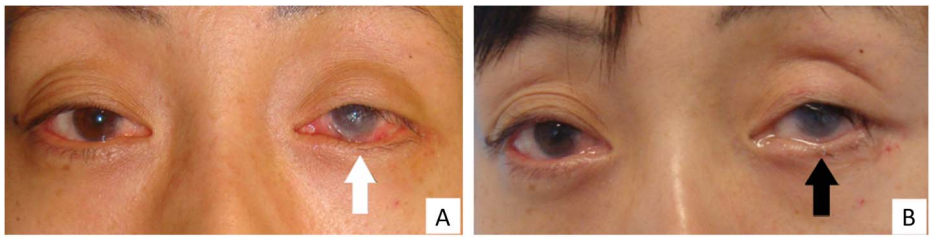

FIGURE 3. A case of successful transplantation (viable gland and unobstructed duct). A, Before transplantation. B, After transplantation. Secretion from the transplanted SMG provided lubrication to the ocular surface structures of the treated eye (black arrow).

In this study, we used the Schirmer test to measure the quantity of secretion from the transplanted glands. The reliability of this test has always been questionable, primarily because irritation from the strip may cause reflex tearing.[22] However, this reflex does not occur in transplanted SMGs. Nonetheless, secretion from transplanted SMGs might increase under conditions of high room temperature or strenuous physical activity.[19] Therefore, we used a modified Schirmer test in which room temperature and physical activity conditions are standardized during examination.[19] The improvement in the Schirmer test value from 0 or 1 to >3 mm indicates a change from "none" to "little," which is more a qualitative change than a quantitative one. Therefore, the improvements in secretion from the transplanted gland measured by the modified Schirmer test are credible.

This study showed that both capsaicin and carbachol increased secretion from transplanted SMGs during the "latent period." Compared with the duct obstruction rate of 18.2% (8/44) in the unexposed group, which did not receive any postoperative intervention, the rate in the exposed group, which received capsaicin and carbachol, was successfully lower at 6.2% (8/128).

In our previous animal experiments, we investigated the mechanism by which capsaicin and carbachol promote salivary secretion from normal and transplanted SMGs. The results showed that TRPV1 and muscarinic receptors and their downstream signaling molecules were downregulated in the early stage after SMG transplantation, and capsaicin and carbachol increased salivary secretion and reduced functional and structural injuries in transplanted SMGs by activating TRPV1 and muscarinic receptor-mediated signal transduction in rabbits.[16,17] Following the tenets of translational medicine, we applied our basic research results to clinical patients. In our subjects as well, we found that administration of capsaicin and carbachol increased salivary secretion, prevented saliva stasis, and finally reduced the rate of duct obstruction in transplanted SMGs. In fact, our previous animal experiments showed that the lack of α- and β-adrenoceptor stimulation was also involved in early dysfunction of the transplanted gland. Phenylephrine (α-adrenoceptor agonist) and isoproterenol (β-adrenoceptor agonist) ameliorated structural injury and improved secretion from the transplanted SMG in rabbits.[23,24] However, considering their potential systemic side effects, the adrenoceptor agonists must be administered through the Wharton duct of the transplanted SMGs, which limits their clinical use.

From our findings, we recommended the combined use of capsaicin and carbachol during the "latent period" as a strategy to prevent duct obstruction in transplanted SMGs, because their effects are different but complementary to each other. Capsaicin mildly increases secretion from transplanted SMGs for over 30 minutes after application and shows a maximum salivary flow rate of 4 mm per 5 minutes. Topical application of capsaicin cream is simple, and patients can do it by themselves. Daily application may be considered to maintain a basic level of secretion from the transplanted gland during the first 3 postoperative months. However, a Schirmer test value of 4 mm indicates a relatively low salivary flow rate. Saliva stasis may occur, followed by mucus embolus formation, as shown in Figure 2. Therefore, carbachol should be administered intermittently as complementary treatment. The salivary flow rate increases greatly after carbachol administration, and this plays the role of internal irrigation to clean viscous depositions out of the duct. However, the effect of carbachol is strong and the injection has to be

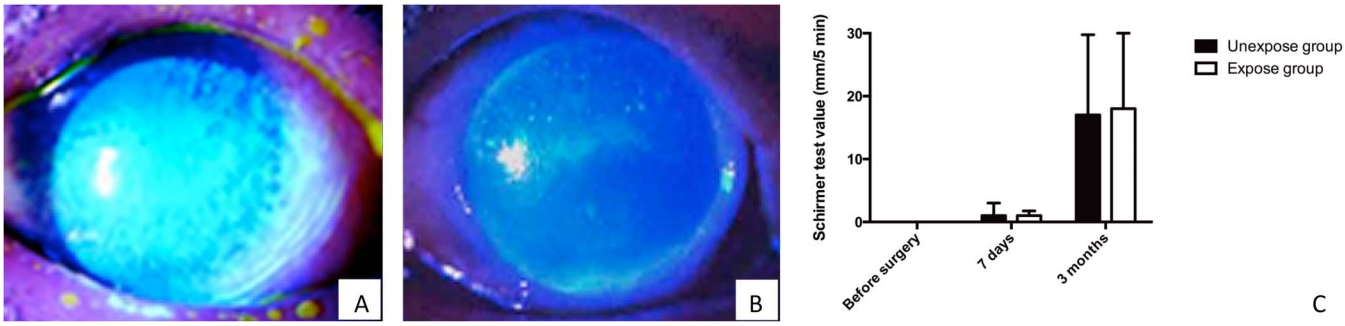

FIGURE 4. Schirmer test value and fluorescent staining of the treated eye. Fluorescent staining on the cornea was less postoperatively. A, Before operation. B, After operation. C, The Schirmer test value improved postoperatively.

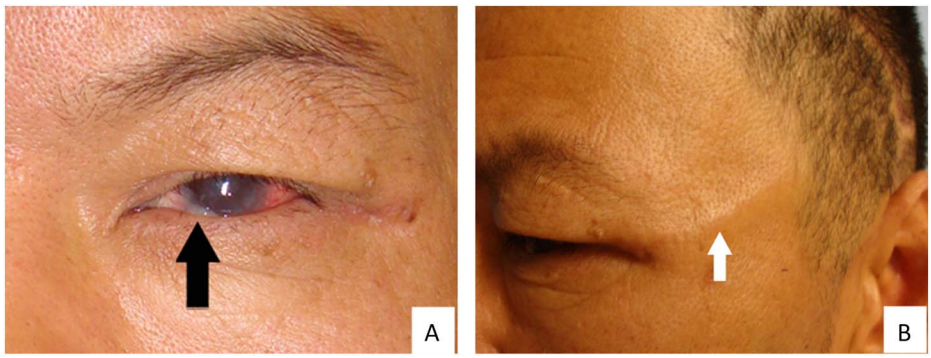

FIGURE 5. A case of duct obstruction. A, Only some sticky secretion was seen in the treated eye (black arrow). B, Dilated duct of a transplanted SMG (white arrow).

administered by a doctor, which makes daily use throughout the "latent period" difficult. We refer to this strategy of combined administration of capsaicin and carbachol as "maintaining continuous secretion with intermittent irrigation."

In the exposed group in this study, 6.2% of patients still experienced duct obstruction in the transplanted SMGs. Clinically, we found that the location of all duct obstructions in the exposed group was the orifice of the Wharton duct. Similarly, 6.8% of patients in the unexposed group also had duct obstructions located in the orifice of the Wharton duct. No difference was found in the rate of duct obstruction in the orifice between the groups ($P = 1$). Ductal orifice obstruction may be caused by scarring of the incision between the Wharton duct orifice and conjunctiva. To prevent scar formation in the ductal orifice, it is essential to harvest the mucosal cuff around the orifice and suture it with the conjunctiva. Usually, the intubated polyethylene tube is left for 7 days in the Wharton duct. For patients lacking the mucosal cuff, this intubated polyethylene tube should be left for more than 1 month to prevent constriction of the orifice due to scar formation. Eleven patients in this study had no mucosal cuff around the orifice of the Wharton duct, and none of them developed duct obstruction in the orifice after delayed withdrawal of the polyethylene tube. However, long-term intubation of the eye is irksome for patients, so it cannot be used commonly.

The low osmolality of secretions from transplanted SMGs compared with natural tears may result in corneal epithelial microcystic edema in patients with excessive saliva and tears.[6,25] An in vitro study showed that natural saliva from the SMG had a severe cytotoxic effect on corneal epithelial cells, and its low osmolality was believed to be the major factor underlying its toxicity.[26] In this study, we found that the high secretory flow rate after carbachol injection was maintained for less than 1 hour, and this temporary increase in secretion from the transplanted SMGs did not cause corneal epithelial edema.

In conclusion, this retrospective study gives evidence that clinical use of capsaicin and carbachol promote secretion from transplanted SMGs. Combined administration of capsaicin and carbachol during the first 3 months postoperatively successfully reduces the rate of duct obstruction in transplanted SMGs. Further prospective studies are required to evaluate this potential treatment.

REFERENCES

1. Lemp MA, Baudouin C, Baum J, et al. The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye Workshop (2007). *Ocul Surf.* 2007;5:75–92.
2. Liu D, Sadhan Y. Surgical punctal occlusion: a prospective study. *Br J Ophthalmol.* 2002;86:1031–1034.
3. Farris RL. Staged therapy for the dry eye. *CLAO J.* 1991;17:207–215.
4. Geerling G, Sieg P, Bastian GO, et al. Transplantation of the autologous submandibular gland for most severe cases of keratoconjunctivitis sicca. *Ophthalmology.* 1998;105:327–335.
5. Qin J, Zhang L, Cai ZG, et al. Microvascular autologous transplantation of partial submandibular gland for severe keratoconjunctivitis sicca. *Br J Ophthalmol.* 2013;97:1123–1128.
6. Borrelli M, Schröder C, Dart JK, et al. Long-term follow-up after submandibular gland transplantation in severe dry eyes secondary to cicatrizing conjunctivitis. *Am J Ophthalmol.* 2010;150:894–904.
7. Jacobsen HC, Hakim SG, Lauer I, et al. Long-term results of autologous submandibular gland transfer for the surgical treatment of severe keratoconjunctivitis sicca. *J Craniomaxillofac Surg.* 2008;36:227–233.
8. Paniello RC. Submandibular gland transfer for severe xerophthalmia. *Laryngoscope.* 2007;117:40–44.
9. Yu GY, Zhu ZH, Mao C, et al. Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca. *Int J Oral Maxillofac Surg.* 2004;33:235–239.
10. Sieg P, Geerling G, Kosmehl H, et al. Microvascular submandibular gland transfer for severe cases of keratoconjunctivitis sicca. *Plast Reconstr Surg.* 2000;106:554–560; discussion 561–552.
11. 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.
12. MacLeod AM, Robbins SP. Submandibular gland transfer in the correction of dry eye. *Aust N Z J Ophthalmol.* 1992;20:99–103.
13. Su JZ, Yang NY, Liu XJ, et al. Obstructive sialadenitis of a transplanted submandibular gland: chronic inflammation secondary to ductal obstruction. *Br J Ophthalmol.* 2014;98:1672–1677.
14. Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic nerves. *Auton Neurosci.* 2007;133:3–18.
15. Ding QW, Zhang Y, Wang Y, et al. Functional vanilloid receptor-1 in human submandibular glands. *J Dent Res.* 2010;89:711–716.
16. Shi L, Cong X, Zhang Y, et al. Carbachol improves secretion in the early phase after rabbit submandibular gland transplantation. *Oral Dis.* 2010;16:351–359.
17. Zhang Y, Cong X, Shi L, et al. Activation of transient receptor potential vanilloid subtype 1 increases secretion of the hypofunctional, transplanted submandibular gland. *Am J Physiol Gastrointest Liver Physiol.* 2010;299:G54–G62.
18. Zhang L, Zhu ZH, Dai HJ, et al. Application of 99m Tc-pertechnetate scintigraphy to microvascular autologous transplantation of the submandibular gland in patients with severe keratoconjunctivitis sicca. *J Nucl Med.* 2007;48:1431–1435.
19. Su JZ, Liu XJ, Zhang L, et al. Schirmer test in transplanted submandibular gland: influencing factors and a modified measurement method. *Cornea.* 2013;32:419–422.

20. Chen ZY, Liang QF, Yu GY. Establishment of a rabbit mode for keratoconjunctivitis sicca. *Cornea*. 2011;30:1024–1029.
21. Geerling G, Garrett JR, Paterson KL, et al. Innervation and secretory function of transplanted human submandibular salivary glands. *Transplantation*. 2008;85:135–140.
22. Kashkouli MB, Pakdel F, Amani A, et al. A modified schirmer test in dry eye and normal subjects: open versus closed eye and 1-minute versus 5-minute tests. *Cornea*. 2010;29:384–387.
23. Li YM, Zhang Y, Shi L, et al. Isoproterenol improves secretion of transplanted submandibular glands. *J Dent Res.* 2009;88:477–482.
24. Xiang B, Zhang Y, Li YM, et al. Effects of phenylephrine on transplanted submandibular gland. *J Dent Res.* 2006;85:1106–1111.
25. Geerling G, Honnicke K, Schröder C, et al. Quality of salivary tears following autologous submandibular gland transplantation for severe dry eye. *Graefes Arch Clin Exp Ophthalmol.* 2000;238:45–52.
26. Geerling G, Daniels JT, Dart JK, et al. Toxicity of natural tear substitutes in a fully defined culture model of human corneal epithelial cells. *Invest Ophthalmol Vis Sci.* 2001;42:948–956.