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Carbachol improves the secretion of transplanted submandibular glands during the latent period after microvascular autologous transplantation for severe keratoconjunctivitis sicca

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Abstract. Poor secretion of transplanted submandibular glands (SMGs) during the latent period may cause duct obstruction and affects the surgical outcome. The objective of this study was to investigate the efficacy and systemic side effects of carbachol on transplanted SMG secretion. Twenty-seven patients who underwent SMG transplantation for severe keratoconjunctivitis sicca were treated with subcutaneous injections of 0.2 mg/2 ml carbachol at 10 days, 1 month, and/or 3 months after surgery. The effect on secretion was evaluated by Schirmer test and technetium 99m (^{99m}Tc) scintigraphy. Systemic side effects were evaluated subjectively using a questionnaire. The results showed that the time to onset varied from 4 to 9 min and the duration of action from 50 to 110 min after carbachol administration. The secretion at each time point after drug administration was significantly higher than the pre-administration value (all $P < 0.01$). ^{99m}Tc scintigraphy showed a decline in the dynamic time–activity curve in 26 patients, demonstrating a stimulatory effect on the secretion of carbachol. No serious systemic side effects were experienced. In conclusion, the intermittent administration of carbachol could be an effective and safe strategy to promote secretion from transplanted SMGs in the latent period to prevent duct obstruction.

Key words: keratoconjunctivitis sicca; dry eye; submandibular gland; submandibular gland transplantation; carbachol; Schirmer test; ^{99m}Tc scintigraphy.

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Keratoconjunctivitis sicca (KCS) is a common disease caused by systemic or optical disorders. Severe KCS can cause corneal ulceration, opacification, or even blindness. Microvascular autologous transplantation of the submandibular gland (SMG) has proved to be an effective treatment for severe cases of KCS.[1–7] Previous work by this team and others has shown that long-term secretory function is retained and the symptoms of KCS are relieved in patients who undergo SMG transplantation procedures.[1,3,5,8,9]

Clinical observations indicate that gland secretion decreases during the first 5–7 days after transplantation, but recovers spontaneously after 3 months. These 3 months include a period of low-level secretion known as the latent period.[1,2,8,10] This period is typically characterized by very low secretion and the occasional secretion of viscous fluid in the resting condition.[1,11] In a pathological process similar to that of obstructive sialadenitis, duct obstruction after SMG transplantation may occur as a result of mucus emboli formed from the trapped viscous fluid.[12,13] This complication can result in insufficient lubrication of the treated eyes.[1] This team has previously reported the incidence of obstructive sialadenitis of the transplanted SMG to be 9.3% within 3 months of transplantation and 1.3% in the long term, i.e., at more than 1 year after surgery.[11] Some patients require reoperation to relieve the obstruction, without which the transplantation is deemed a failure.[1]

To improve surgical outcomes and the quality of life of patients with severe KCS undergoing autologous SMG transplantation, an intervention during the latent period is of critical importance.

Normal SMG secretion is controlled by cholinergic parasympathetic and sympathetic nerves.[14–17] However, both of these nerve types are incised during transplantation; thus, the transplanted SMG is completely denervated. Some studies have confirmed that the parasympathetic and sympathetic nerve fibres are restored in the long term, thereby demonstrating that reinnervation occurs[5]; however, reinnervation-induced secretion is not found during the latent period.[18]

Using a rabbit model of SMG transplantation, this team has previously shown that in the absence of acetylcholine, the expression levels of the muscarinic acetylcholine receptors M1 and M3 are reduced in the early stages after surgery.[19] In addition, carbachol has been found to increase salivary secretion and prevent functional and structural injuries of the transplanted SMGs by increasing M1 and M3 receptor expression.[19] These findings indicate that carbachol treatment could serve as a novel clinical strategy to improve secretion and prevent duct obstruction of the transplanted SMGs during the latent period.

Carbachol is a commercially available parasympathetic mimetic agent that is commonly administered via retrobulbar injection during eye surgery, or via systemic injection to treat uroschesis, postoperative abdominal distension, and xerostomia. van Acker et al. used carbachol to stimulate salivary gland secretion during single photon emission computed tomography (SPECT) to determine the relationship between radiation dose and salivary gland dysfunction after radiotherapy.[20] Geerling et al. also suggested the use of carbachol to stimulate gland secretion during salivary gland scintigraphy for transplanted SMGs.[5] However, no studies have reported the use of carbachol to promote SMG secretion and to prevent obstructive sialadenitis of the transplanted SMGs.

The objectives of this study were to investigate whether carbachol could promote the secretion of transplanted SMGs during the latent period via systemic administration and to identify its systemic side effects, if any.

Materials and methods

Twenty-seven patients (11 men and 16 women) with a mean age of 37.5 years (range 17–51 years) who underwent autologous SMG transplantation between May 2008 and December 2012 were enrolled in this study. The aetiologies of KCS were Stevens–Johnson syndrome in 18 patients, acute conjunctivitis in three patients, corneal pemphigoid in one patient, corneal chemical burns in one patient, and unknown in four patients. KCS status was evaluated by an ophthalmologist using the Schirmer test, break-up time, and fluorescence staining.[1] The indications for SMG transplantation included the following: (1) obvious, persistent symptoms of dry eye and failure of other ophthalmological treatments, and (2) Schirmer test result <2 mm/5 min, break-up time result <5 s, and fluorescence staining (score value ≥ 10) on ophthalmological evaluation. Contradictions to SMG transplantation included (1) obvious symptoms of xerostomia or Sjögren syndrome, (2) Schirmer test result >5 mm/5 min, and (3) hypofunction of multiple major salivary glands on scintigraphy.[1]

No control group was set in this study for two reasons. Firstly, in the authors' long term clinical experience, secretion in the latent period is very low and the patient's general physical condition rarely affects the secretion from the transplanted SMG. Secondly, results from animal studies and a pilot study on patients found that carbachol was very effective on the secretion of the transplanted SMG and therefore very unlikely to be affected by other factors, if any exist. The study was approved by the necessary institutional review board. All of the patients signed an informed consent form before treatment.

Administration of carbachol

All patients were injected subcutaneously with 0.2 mg/2 ml carbachol (Shandong Bausch and Lomb Freda Pharmaceutical Co., Ltd, Jinan, China) bilaterally in the abdomen, 5 cm below the navel, on day 10 postoperative and at 1 month and/or 3 months after autologous SMG transplantation. All patients were subject to electrocardiographic monitoring during carbachol administration. Atropine was available for administration in the event of severe cardiovascular symptoms or other severe systemic side effects.

No carbachol injection was given if the patient's resting Schirmer test result was >10 mm/5 min or if epiphora was reported; this was set as the endpoint of the treatment.

Schirmer test procedure

A Schirmer test was performed before (baseline) and at 5, 10, 15, 30, and 60 min after drug administration. The room temperature was maintained at 23 °C and patients were asked to refrain from any physical activity or glandular stimulation for 30 min to prevent these affecting the flow rate.[21] When the secretion rate was moderate, it was measured according to a standard Schirmer test protocol with no anaesthesia. The bent end of a Whatman No. 41 paper strip (5 mm × 120 mm) was inserted into the lateral side of the lower conjunctival fornix for 5 min and the length of the wet strip was recorded.[21] When the secretion rate was high, the fluid secreted in 5 min was collected in Eppendorf tubes. The collected salivary tears were then dropped onto a Whatman paper strip using a pipette slowly and evenly. The length of the wet strip was recorded. The values were recorded as ST1 (baseline) and ST2–ST6 for the subsequent time points. A stop watch was used to record the onset time and end point. Onset time was defined as the time when visible salivary secretion could be seen from the conjunctival sac.

Additionally, beyond 60 min, the secretion from the transplanted SMG was observed. When it appeared to subside, it was tested continuously until it was less than or equal to that at baseline. The time taken to achieve this was recorded as the duration of action.

Evaluation by technetium 99m (^{99m}Tc) scintigraphy

In addition to the Schirmer test, ^{99m}Tc scintigraphy was used to evaluate the efficacy of carbachol before surgery and at 7 days and at 3 months after surgery.[18] ^{99m}Tc scintigraphy was performed using Hawkeye SPECT equipment (GE Healthcare, Tirat Hacarmel, Israel). Patients fasted for at least 8 h before the examination. During the whole procedure, patients were positioned using a radiolucent and neck-contoured headrest made of plastic. After intravenous injection of the tracer ^{99m}Tc pertechnetate, sequential images were acquired for 30 min at a rate of 1 min/frame using a gamma camera. At 15 min, 0.2 mg/2 ml carbachol was administered subcutaneously. Throughout the study, the camera was fitted with a low-energy ultra high resolution (LEUHR) collimator. Regions of interest (ROIs) were drawn by overlaying all frames of transplanted SMGs and the contralateral SMGs, and the background ROI was marked in the frontal region.[18] Dynamic time–activity curves were generated automatically by the workstation after all ROIs were drawn (Fig. 1).

Three parameters were used to investigate the secretion efficiency of transplanted SMGs in response to carbachol, namely the uptake ratio (UR), salivary excretion fraction (SEF), and latent time. UR was defined as the ratio between maximum radiation counts and the background radiation counts. The UR is a measure of the uptake function of acinar cells. The SEF is defined as the ratio of the difference between the maximum and minimum radiation counts to the maximum radiation counts. It is a measure of the stimulatory effect of carbachol on acinar cells. The latent time is defined as the time interval between carbachol administration and excretion, and it represents the speed at which acinar cells respond to carbachol. As latent time is affected by various parameters, such as the thickness of the subcutaneous fat tissue, heart rate, injection rate, etc., the latency index was also obtained. The latency index is the ratio between the onset time of transplanted SMGs and the average onset time of bilateral parotid glands.

Radiation counts for maximum and minimum activity after carbachol injections were denoted as 'a' and 'b', radiation counts in the background area were denoted as 'c'. The time point of the maximum radiation count was Ta. Accordingly, the UR, SEF, latent time, and latent index of the transplanted SMGs were calculated as follows:

$$\mathrm{UR} = (\mathrm{a}-\mathrm{c})/\mathrm{c},$$

$$\mathrm{SEF} = (\mathrm{a}-\mathrm{b})/(\mathrm{b}-\mathrm{c}),$$

$$\mathrm{T_{latent}} = \mathrm{Ta}-15,$$

$$\text{Latent index} = 2\times \mathrm{T_{latent\ transplanted\ SMG}}/\left(\mathrm{T_{latent\ left\ parotid\ gland}} + \mathrm{T_{latent\ right\ parotid\ gland}}\right)$$

Systemic side effects questionnaire

A subjective questionnaire was used to investigate the occurrence of systemic side effects. The questionnaire was designed to obtain patient responses related to drug side effects, including salivary secretion, lacrimal secretion, gastrointestinal peristalsis, sweating, abdominal pain, headache, agitation, and bladder filling. Each side effect was categorized into one of three grades, namely, mild, moderate, and severe. The classification criteria are listed in Table 1. Patients were asked to return the completed questionnaire immediately after treatment at each time point.

Statistical analysis

The designated normality test was used to check whether or not the results of each parameter were normally distributed. If the data were normally distributed, they were expressed as the mean ± standard deviation (SD) value; if not, they were expressed as the median (quartile) value. Results at ST2–ST6 were compared with those at ST1 using Dunnett's test to determine the effect of carbachol on the secretion efficiency of transplanted SMGs. Postoperative UR, SEF, and latency index were compared with preoperative values using Dunnett's test. $P < 0.05$ was considered to be statistically significant.

Results

All 27 patients received carbachol treatment at 10 days and within 1 month of transplantation. There were five patients whose resting secretion value was

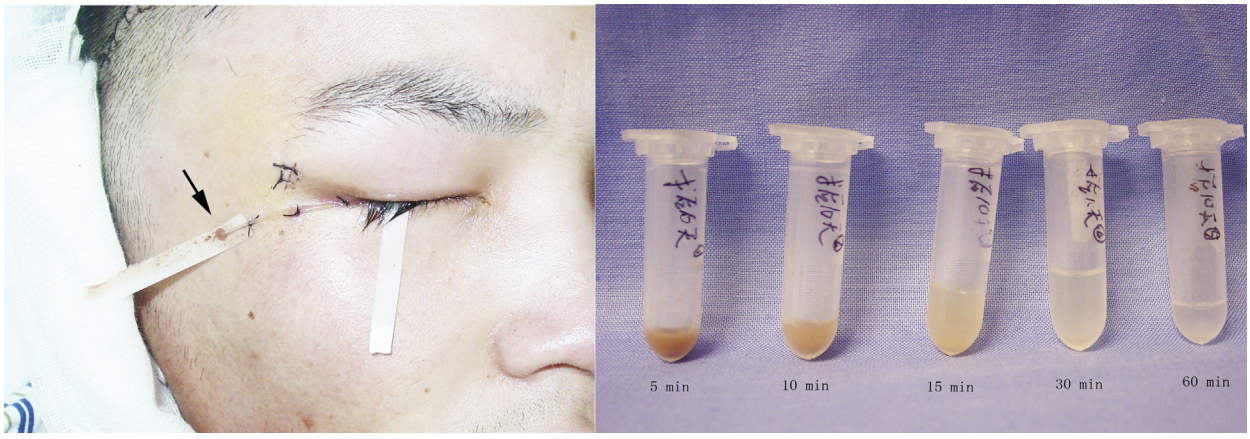

Fig. 1. Dynamic time–activity curves obtained after carbachol administration. Left panel: Serial scintigram showing ^{99m}Tc-pertechnetate uptake by a transplanted submandibular gland (arrowhead). Right panel: The blue line represents the dynamic time–activity curve of the transplanted SMG. It shows a decline in the activity of a transplanted SMG after carbachol administration (↓). Point A is the maximum activity after injection, point B is the minimum activity after injection, and point C is the background activity. The pink line represents the dynamic time–activity curve of the contralateral SMG, which also shows a decline in activity after carbachol administration. The green line represents the background in the frontal area, which shows no response to carbachol administration.

Table 1. Classification criteria for systemic side effects.

	Mild	Moderate	Severe
Over-secretion of saliva	Moisture in the mouth	Need to swallow from time to time	Swallow constantly
Lacrimal secretion	Moisture in the eye	Tears produced when closing the eyes	Epiphora
Gastrointestinal peristalsis	Quick gastrointestinal motility	Bowel sounds can be heard by the doctor	Exhaust constantly
Nausea and vomiting	Nausea, but bearable	Nausea, unbearable	Vomiting
Sweating	Skin moist	Small amount of sweat	Sweating
Abdominal pain	Yes, but bearable	Unbearable	–
Headache	Yes, but bearable	Unbearable	–
Agitation	Yes, but bearable	Unbearable	–
Bladder filling	Desire to void	Bladder fullness	Urgency of urination

>10 mm at 3 months. Consequently, they did not receive carbachol injections. The other 22 patients received carbachol treatment at 3 months.

A strong stream of salivary tears was found to wash out the thrombotic and mucus emboli from the ducts (Fig. 2). The salivary tears shed in the first 10 min were thick and 'muddy', but cleared over time. Thus, carbachol served as a facilitator of 'internal irrigation'.

Schirmer test

The time to onset after carbachol subcutaneous injection varied from 4 to 9 min. The duration of action varied from 50 to 110 min after carbachol administration. Detailed results are shown in Table 2. Since the results of the Schirmer test were not all normally distributed, they were expressed as the median (quartile) (Table 3). Values at ST2, ST3, ST4, ST5, and ST6 were all significantly higher than the value at ST1 ($P < 0.01$).

^{99m}Tc scintigraphy

^{99m}Tc scintigraphy was performed before transplantation, at 7 days after transplantation, and at 3 months after transplantation for all 27 patients. A decline in the dynamic time–activity curve, which means a stimulatory effect on the secretion of carbachol, was shown in 26 patients at both time points (Fig. 1). One patient showed no decline in postoperative dynamic time–activity curve at either time point (Fig. 3).

Table 4 shows the UR, SEF, and latent index values obtained before surgery and at 7 days and 3 months after surgery. The UR, SEF, and latent index results were normally distributed and were thus expressed as the mean ± standard deviation (SD). However, the latent time results were not normally distributed and were expressed as the median (quartile). Compared to the preoperative values, UR and SEF values were significantly higher at 7 days ($P < 0.01$, $P < 0.01$) and at 3 months ($P < 0.05$, $P < 0.05$) after transplantation. However, the latent index values at 7 days and at 3 months were both significantly lower than the preoperative values ($P < 0.05$).

Systemic side effects

During the treatment, 76 doses of carbachol were administered ($n = 27$ at 10 days, $n = 27$ at 1 month, and $n = 22$ at 3 months after surgery). The blood pressure and heart rate of all patients were stable during the treatment period. The top three side effects were a mild increase in salivary secretion (59.2% of patients), mild sweating (47.4% of patients), and a mild increase in gastrointestinal peristalsis (13.2% of patients). Other systemic side effects are shown in Table 5.

Discussion

Salivary gland secretion is mainly regulated by parasympathetic autonomic nerves via the activation of muscarinic

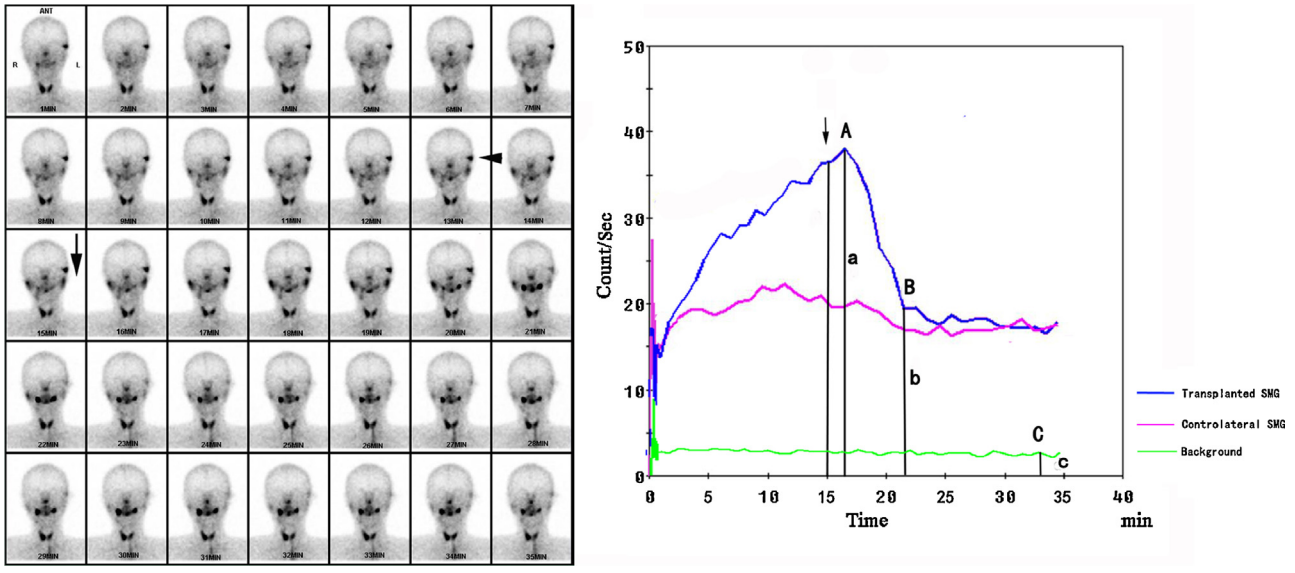

Fig. 2. Secretion from the transplanted SMG after carbachol administration. Left panel: Washout of the thrombotic and mucus emboli by a strong stream of saliva tears from Wharton's duct of the transplanted SMG (arrow). Right panel: Salivary tears secreted from a transplanted SMG every 5 min. The secretions obtained during the first 10 min were thick and 'muddy', but the secretions became clearer at later time points.

Table 2. The time to onset of action and the duration of action of carbachol injections at the different postoperative time points; mean ± SD.

Time	Number of patients	Time to onset, min	Duration of action, min
10 days	27	6.0 ± 2.1	80.0 ± 23.7
1 month	27	5.9 ± 1.3	61.8 ± 12.8
3 months	22	8.7 ± 0.2	86.7 ± 24.3

SD, standard deviation.

acetylcholine receptors (mAChRs),[14,16] especially M1 and M3.[15] Based on this mechanism, pilocarpine, an agonist of mAChRs, has been used successfully to treat xerostomia[22,23] and obstructive sialadenitis.[13]

Because of their denervation, the secretion mechanism of transplanted SMGs is altered. The very low secretion during the latent period may cause duct obstruction. In order to identify a clinically effective, safe, and convenient method to improve glandular secretion in the latent period, this team has investigated the regulation of denervated SMGs in a rabbit model of SMG transplantation during the early stages after surgery. The expression levels of M1 and M3 receptors and the signalling of their downstream target molecules decreased in the early stages after SMG transplantation, and this down-regulation was associated with reduced secretion.[19] In this model, carbachol increased salivary secretion and reduced the functional and structural injuries of the transplanted SMGs by activating M3 receptor-mediated signal transduction.[19]

In the present study, it was attempted to translate these basic research findings to the clinical setting. The results of the present study demonstrated that subcutaneously injected carbachol elicits a rapid and strong response in terms of the secretion of salivary tears. The response begins at 3–5 min after administration and lasts for ≥1 h. The average peak secretion was found to be >200 mm (Schirmer test), and the SEF approached 0.5 (^{99m}Tc scintigraphy). This 'internal irrigation' was able to wash out the mucus and thrombotic emboli and prevent duct obstruction. Furthermore, the systemic administration of carbachol was found to be safe; no serious systemic side effects occurred. Echocardiography did not reveal any cardiovascular abnormalities. Taken together, carbachol was found to safe and efficacious in improving salivary secretion during the latent period.

It was found that with increased time following the operation, there was a delayed onset of action for carbachol. With ^{99m}Tc scintigraphy, the postoperative latent time decreased when compared to the preoperative time. This indicates that the transplanted SMG is more sensitive to carbachol following transplantation. Studies on denervation of the autonomic nervous system have shown that the destruction and degeneration of preganglionic or postganglionic autonomic axons is followed by greatly increased sensitivity of the denervated autonomic

Table 3. Secretion of transplanted SMGs before and after carbachol administration; median (quartile).[a]

Time after surgery	Number of patients	ST1, mm	ST2, mm	ST3, mm	ST4, mm	ST5, mm	ST6, mm
10 days	27	1.5 (0.5)	150.5 (216.0)[b]	168.0 (216.0)[b]	161.5 (216.0)[b]	128.5 (174.5)[b]	103.5 (174.5)[b]
1 month	27	1.5 (0.5)	338.0 (243.0)[b]	338.0 (277.0)[b]	282.5 (176.0)[b]	91.5 (129.5)[b]	66.5 (48.0)[b]
3 months	22	9.5 (0.5)	589.5 (161.0)[b]	675.0 (451.5)[b]	257.0 (166.0)[b]	73.3 (108.0)[b]	46.5 (71.5)[b]

SMG, submandibular gland.

[a] The Schirmer test (5 min) was performed before (ST1) and at 5 (ST2), 10 (ST3), 15 (ST4), 30 (ST5), and 60 (ST6) min after carbachol administration.

[b] Significantly different compared to ST1 level ($P < 0.01$).

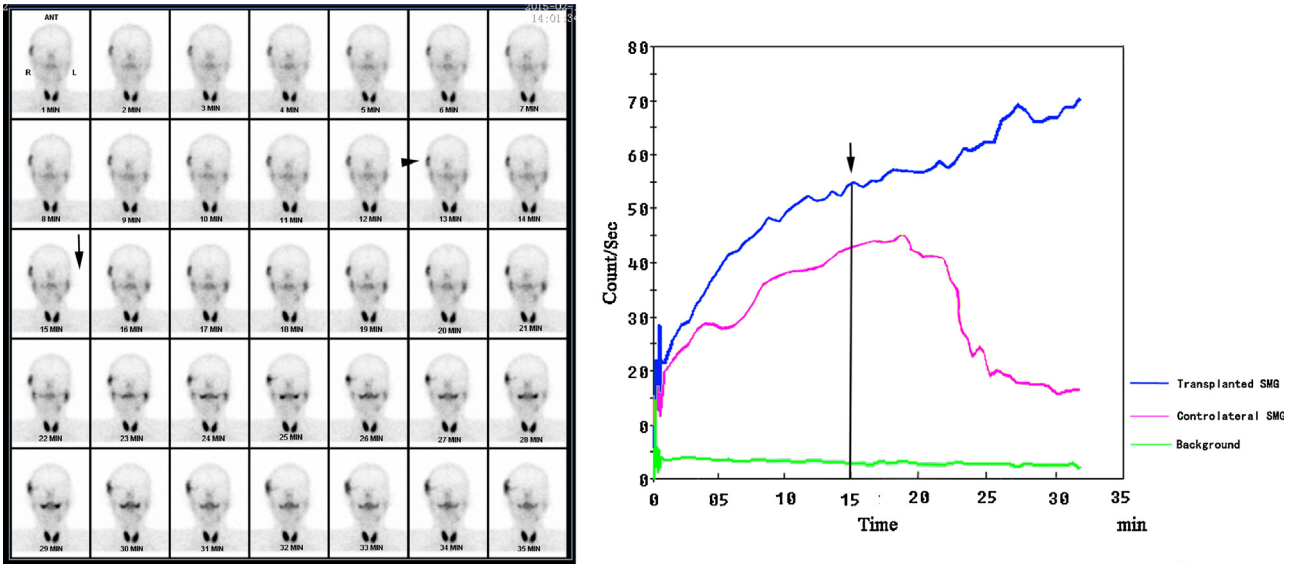

Fig. 3. Dynamic time–activity curve obtained after carbachol administration in a single patient. Left panel: Serial scintigram showing ^{99m}Tc-pertechnetate uptake by a transplanted SMG (arrowhead). Right panel: The blue line represents the dynamic time–activity curve of the transplanted SMG. It does not show any decline in activity after carbachol administration (↓). The pink line represents the dynamic time–activity curve of the contralateral SMG, which shows a decline in activity after carbachol administration. The green line represents the background in the frontal area, which shows no response to carbachol administration.

Table 4. Comparisons of ^{99m}Tc scintigraphy indices between preoperative levels and those obtained at 7 days and 3 months postoperative.

Timing	Number of patients	UR	SEF	Latent time[a], min	Latent index
Preoperative	27	7.5 ± 2.8	0.2 ± 0.1	3.7 (3.8)	1.7 ± 0.3
7 days	26	11.2 ± 4.2[c]	0.4 ± 0.1[c]	3.3 (2.2)[b]	0.5 ± 0.4[b]
3 months	26	10.0 ± 3.1[b]	0.4 ± 0.1[b]	2.3 (3.0)[b]	0.9 ± 0.1[b]

UR, uptake ratio; SEF, salivary excretion fraction.
[a] The data were not normally distributed; the results are presented as the median (quartile).
[b] Significantly different compared to the preoperative level, $P < 0.05$.
[c] Significantly different compared to the preoperative level, $P < 0.01$.

Table 5. Incidence of systemic side effects of carbachol ($N = 76$[a]).

	None, *n* (%)	Mild, *n* (%)	Moderate, *n* (%)	Severe, *n* (%)
Over-secretion of saliva	28 (36.8)	45 (59.2)	3 (3.9)	0
Lacrimal secretion	76 (100)	0	0	0
Gastrointestinal peristalsis	63 (82.9)	10 (13.2)	2 (2.6)	1 (1.3)
Nausea and vomiting	67 (88.2)	9 (11.8)	0	0
Sweating	29 (38.2)	36 (47.4)	8 (10.5)	3 (3.9)
Abdominal pain	76 (100)	0	0	0
Headache	74 (97.4)	2 (2.6)	0	0
Agitation	74 (97.4)	2 (2.6)	0	0
Bladder filling	75 (98.7)	1 (1.3)	0	0

[a] $n = 27$ at 10 days after surgery, $n = 27$ at 1 month after surgery, and $n = 22$ at 3 months after surgery.

effectors to their specific neurotransmitters and to pharmacological agonists.[24,25] Emmelin described the relationship between the paralytic secretion of saliva and super-sensitivity after denervation.[26] This helps explain why with systemic administration, carbachol has powerful effects on transplanted SMGs, while it has little effect on other target organs.

The findings of this study are also of relevance for guiding modifications to the technique of salivary gland ^{99m}Tc scintigraphy. ^{99m}Tc scintigraphy plays an important role in preoperative evaluation and decision-making before autologous SMG transplantation.[18] Because the transplanted SMGs are denervated and not responsive to citric acid, no decline occurs in the dynamic time–activity curves using the typical ^{99m}Tc scintigraphy protocol. Thus, the function of the transplanted SMGs cannot be determined quantitatively by scintigraphy. Moreover, imaging of the transplanted SMG duct is difficult to complete within 25 min. Generally, 120–180 min is required to show drainage of ^{99m}Tc-pertechnetate into the orbit through the ducts of transplanted SMGs and subsequently into the nasolacrimal duct and the nasal cavity. The image thus obtained is called a delayed Image 18. Therefore, the time spent on diagnosis of duct obstruction increases. The results of the present study demonstrate that the secretion of transplanted SMGs could be stimulated using carbachol during ^{99m}Tc scintigraphy, even in the very early stages after transplantation. Thus, imaging could be completed within 25 min.

With the obvious excretion response on dynamic time–activity curves, certain commonly used parameters such as the UR and SEF could be calculated, and the function of transplanted SMGs could be analyzed as that of normal salivary glands. However, normal values should be collected using the same protocol before the method can be used routinely.

In conclusion, subcutaneous injection of carbachol at a dose of 0.2 mg/2 ml could be an effective and safe method to promote the secretion of transplanted SMGs in the latent period after surgery.

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Competing interests

None.

Ethical approval

The study was approved by the Institutional Review Board of the Peking University Health Science Centre (No. IRB00001052-08048).

Patient consent

Written patient consent was obtained for publication of the clinical photographs.

References

1. Yu GY, Zhu ZH, Mao C, Cai ZG, Zou LH, Lu L, et al. Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca. *Int J Oral Maxillofac Surg* 2004;**33**:235–9.
2. 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. discussion 561–2.
3. Qin J, Zhang L, Cai ZG, Mao C, Liu XJ, Lv L, et al. Microvascular autologous transplantation of partial submandibular gland for severe keratoconjunctivitis sicca. *Br J Ophthalmol* 2013;**97**:1123–8.
4. Paniello RC. Submandibular gland transfer for severe xerophthalmia. *Laryngoscope* 2007;**117**:40–4.
5. Geerling G, Garrett JR, Paterson KL, Sieg P, Collin JR, Carpenter GH, et al. Innervation and secretory function of transplanted human submandibular salivary glands. *Transplantation* 2008;**85**:135–40.
6. 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.
7. Borrelli M, Schroder C, Dart JK, Collin JR, Sieg P, Cree IA, 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.
8. 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.
9. 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.
10. Ding C, Cong X, Zhang Y, Yang NY, Li SL, Wu LL, et al. Hypersensitive mAChRs are involved in the epiphora of transplanted glands. *J Dent Res* 2014;**93**:306–12.
11. Su JZ, Yang NY, Liu XJ, Cai ZG, Lv L, Zhang L, et al. Obstructive sialadenitis of a transplanted submandibular gland: chronic inflammation secondary to ductal obstruction. *Br J Ophthalmol* 2014;**98**:1672–7.

12. Pace CG, Hwang KG, Papadaki M, Troulis MJ. Interventional sialoendoscopy for treatment of obstructive sialadenitis. *J Oral Maxillofac Surg* 2014;**72**:2157–66.
13. Al-Nawas B, Beutner D, Geisthoff U, Naujoks C, Reich R, Schroder U, et al. [The new S2k AWMF guideline for the treatment of obstructive sialadenitis in commented short form]. *Laryngorhinootologie* 2014;**93**:87–94.
14. Ryberg AT, Warfvinge G, Axelsson L, Soukup O, Gotrick B, Tobin G. Expression of muscarinic receptor subtypes in salivary glands of rats, sheep and man. *Arch Oral Biol* 2008;**53**:66–74.
15. 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.
16. Gautam D, Heard TS, Cui Y, Miller G, Bloodworth L, Wess J. Cholinergic stimulation of salivary secretion studied with M1 and M3 muscarinic receptor single- and double-knockout mice. *Mol Pharmacol* 2004;**66**:260–7.
17. Baum BJ. Principles of saliva secretion. *Ann N Y Acad Sci* 1993;**694**:17–23.
18. Zhang L, Zhu ZH, Dai HJ, Cai ZG, Mao C, Peng X, 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**:1431–5.
19. Shi L, Cong X, Zhang Y, Ding C, Ding QW, Fu FY, et al. Carbachol improves secretion in the early phase after rabbit submandibular gland transplantation. *Oral Dis* 2010;**16**:351–9.
20. van Acker F, Flamen P, Lambin P, Maes A, Kutcher GJ, Weltens C, et al. The utility of SPECT in determining the relationship between radiation dose and salivary gland dysfunction after radiotherapy. *Nucl Med Commun* 2001;**22**:225–31.
21. 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.
22. Wynn RL, Meiller TF. Artificial saliva products and drugs to treat xerostomia. *Gen Dent* 2000;**48**:630–6.
23. Almeida JP, Kowalski LP. Pilocarpine used to treat xerostomia in patients submitted to radioactive iodine therapy: a pilot study. *Braz J Otorhinolaryngol* 2010;**76**:659–62.
24. Falzon K, Jungkim S, Charalampidou S, Townley D, Flitcroft DI. Denervation supersensitivity to 1% phenylephrine in Horner syndrome can be demonstrated 10 days after the onset of symptoms. *Br J Ophthalmol* 2009;**93**:130.
25. Kaseda S, Zipes DP. Supersensitivity to acetylcholine of canine sinus and AV nodes after parasympathetic denervation. *Am J Physiol* 1988;**255**:H534–9.
26. Emmelin N. Paralytic secretion of saliva: an example of supersensitivity after denervation. *Physiol Rev* 1952;**32**:21–46.

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