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The timing of acid-induced increase in saliva secretion in transplanted submandibular glands

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Abstract. The purpose of this study was to determine the timing of acid-induced increase in saliva secretion and to investigate the possibility of parasympathetic reinnervation of transplanted submandibular glands (SMGs). Citric acid stimulation-induced changes in secretion of transplanted SMGs were evaluated in 27 patients who underwent SMG transplantation for keratoconjunctivitis sicca (KCS); ^{99m}Tc scintigraphy and Schirmer tests were done at 1, 3, 6, and 9 months after transplantation. Acetylcholinesterase staining was conducted to confirm the presence of parasympathetic reinnervation in three SMGs at 6 and 9 months after transplantation. Schirmer tests showed significantly increased secretion of the transplanted SMGs after acid stimulation at 6 and 9 months, but not at 1 and 3 months. On ^{99m}Tc scintigraphy, no decline was detected on the dynamic time–activity curve after acid stimulation at 1 and 3 months, but a decline was detected in nine glands at 6 months and in 19 glands at 9 months. No decline was observed in the remaining eight glands at 9 months after transplantation. The histology findings were consistent with scintigraphy results. In conclusion, acid-induced increase in saliva secretion occurs at ≥6 months after SMG transplantation, and parasympathetic reinnervation of the transplanted SMG might occur.

Key words: keratoconjunctivitis sicca; submandibular gland transplantation; reinnervation; scintigraphy; Schirmer test.

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Keratoconjunctivitis sicca (KCS), also known as dry eye syndrome, is a common disease caused by systemic or optical disorders. Severe dryness of the eyes can result in corneal ulceration, corneal opacification, and even blindness. Autologous microvascular transplantation of the submandibular gland (SMG) has proven to be an effective treatment for patients with severe KCS.[1–10] In our experience of SMG transplantation in 180 patients (195 glands), all viable transplanted SMGs retain their secretion function in the long term and the symptoms of dry eye are greatly relieved.[3,8] However, excessive secretion, or epiphora, occurs in more than 40% of patients at >6 months after surgery.[8] Patients with severe epiphora may experience mild discomfort at rest and at room temperature, but may experience worsened symptoms upon exercise or at increased temperatures. These

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patients may require reduction surgery of the transplanted SMG.

Secretion from the SMG is controlled by both parasympathetic and sympathetic nerves. However, the secretory mechanism of the transplanted SMG is different from that of the normal SMG because the nerves supplying the gland are severed during the transplantation procedure and the glands become denervated. Parasympathetic denervation of the transplanted SMG has been shown in a previous study by our group, in which ^{99m}Tc pertechnetate scintigraphy was performed in the early postoperative period (<3 months) after SMG transplantation.[10] In that study, it was found that the uptake of ^{99m}Tc pertechnetate in the transplanted gland was not influenced by acid stimulation and increased slowly and steadily.[10] Upon long-term (>6 months postoperative) observation, it was found that secretion from transplanted SMGs could be promoted by sour food in about 60% of patients.

A series of studies on specimens harvested during reduction surgery has found that muscarinic acetylcholine receptors 1 and 3 (M1- and M3-mAChRs) are upregulated in transplanted SMGs obtained from epiphora patients. The hypersensitive mAChRs would then induce AQP5 trafficking out of lipid rafts, resulting in excessive gland secretion.[11] Geerling et al.[12] demonstrated the distribution of autonomic nerve endings in transplanted SMGs in the long term. These fundamental research studies indicate the possibility of reinnervation of the transplanted glands.

However, the timing of acid-induced increase in saliva secretion and its correlation with the parasympathetic reinnervation of transplanted SMGs remain unknown. The purpose of this study was to determine the timing of acid-induced increase in saliva secretion and to investigate the possibility of parasympathetic reinnervation of transplanted SMGs.

Materials and methods

This study involved 27 consecutive patients (11 men, 16 women; mean age 37.5 years, range 17–51 years) who underwent autologous SMG transplantation between May 2008 and December 2012. The main cause of severe dry eye was Stevens–Johnson syndrome (18 patients), followed by chronic keratoconjunctivitis (three patients), corneal pemphigoid (one patient), and chemical burn of the cornea (one patient). The cause was unknown for four patients. Before surgery, detailed ophthalmological, oral, and maxillofacial evaluations were done and ^{99m}Tc pertechnetate scintigraphy was carried out following a previously established protocol.[3,10] 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 <2 mm, break-up time <5 s, and positive fluorescence staining on ophthalmological evaluation. The contradictions included (1) obvious symptoms of xerostomia or Sjögren syndrome, (2) Schirmer test >5 mm, and (3) hypofunction of multiple major salivary glands on scintigraphy.[3] The study was approved by the Ethics Committee for Human Experiments of Peking University.

All transplanted SMGs were confirmed to be viable by ^{99m}Tc pertechnetate scintigraphy performed 7 days after the surgery.[11] The patients were followed up at 1, 3, 6, and 9 months after the surgery. Parasympathetic stimulation was induced by oral instillation of 0.5 ml of 2.5% citric acid. During each follow-up visit, the patients underwent ^{99m}Tc scintigraphy and the Schirmer test. The effect of parasympathetic innervation on the secretion of the transplanted SMG was evaluated by comparing the secretion function before and after citric acid stimulation via both methods.

Schirmer test

The Schirmer test was performed using Whatman No. 41 paper (5 mm × 120 mm). The bent end of the paper was inserted into the lateral side of the lower conjunctival fornix, without using any anaesthesia. When the total length of the strip was moistened within 5 min, the secretion rate was considered high, and the test was carried out for a further 1 min with a fresh strip. The results were calculated for 5 min by multiplying the length of paper strip moistened in 1 min by a factor of three, according to the method reported by Jones et al.[13] When the secretion rate was moderate, the test was performed for 5 min.

The Schirmer test was performed in the resting condition and in the acid-stimulated condition, with a minimum interval of 30 min between the two. In the resting condition, the room temperature was 23 °C and the patients rested for 30 min without any physical activity or glandular stimulation before the test.[14] This test was conducted once, and the result was recorded as ST1. In the acid-stimulated condition, the room temperature was 23 °C. No glandular massage was administered, but 0.5 ml of 2.5% citric acid was instilled orally on the anterior part of the dorsum of the tongue. For each patient, a total of three Schirmer tests (5 min per test) were conducted immediately after acid instillation. The results were recorded as ST2, ST3, and ST4, respectively.

^{99m}Tc scintigraphy

Scintigraphy was carried out using Hawkeye SPECT equipment (General Electric Healthcare, USA). Patients fasted for at least 8 h before the examination. During the whole acquisition, the patient's head was fixed in place using a radiolucent plastic neck-contoured headrest. After intravenous injection of the tracer (^{99m}Tc pertechnetate), sequential images at 1 min/frame were acquired for 30 min using a gamma camera. At 15 min, 0.5 ml of 2.5% citric acid was instilled orally on the anterior part of the dorsum of the tongue with a syringe. Throughout the test, the camera was fitted with a low-energy, ultra-high-resolution collimator. Data were stored in a 128 × 128 matrix with 2.3 zoom and a pixel size of 5 mm. Regions of interest were drawn over the transplanted SMG in all of the frames, and the background region of interest was marked in the frontal region.[10] A dynamic time–activity curve was generated automatically by the workstation after all the regions of interest were drawn.

Histology and acetylcholinesterase staining

Three of the 27 patients developed severe epiphora after the transplantation surgery, one at 6 months and two at 9 months. In these patients, the secretion function of the transplanted SMG was measured using the Schirmer test with and without citric acid stimulation and by ^{99m}Tc scintigraphy. The patients underwent reduction surgery of the transplanted SMG to control the severe epiphora. Surgical specimens from these three patients were examined histologically and stained for acetylcholinesterase to observe the density of parasympathetic nerve endings. One normal SMG, which was harvested from a patient with primary oral squamous cell carcinoma who underwent elective neck dissection without irradiation or chemotherapy, was used as a control gland. The glandular tissue was confirmed to be histologically normal by an experienced pathologist.

Parts of the SMG specimens were fixed in formaldehyde and embedded in paraffin wax. The sections were then cut and stained with haematoxylin and eosin.