Original article

Establishment of submandibular gland allotransplantation model in miniature swine

GE Xi-yuan, YU Guang-yan, CAI Zhi-gang and MAO Chi

Background Autologous transplantation of the submandibular gland (SMG) into the temporal fossa with microvascular anastomosis has been successfully applied in severe xerophthalmia patients as a permanent tear substitute. However, severe xerophthalmia can be accompanied by salivary gland dysfunction, making such autotransplantation unsuitable. Therefore, SMG allotransplantation might be a solution. The aim of this study was to assess the technical feasibility of submandibular gland allotransplantation.

Methods Twelve miniature swine were randomized to serve as donors or recipients. One SMG was transplanted between a donor and a recipient. The donor SMG was revascularized by microvascular anastomosis of its vascular pedicle to the recipient lingual artery and external jugular vein. The secretory duct was implanted into the vestibule of the mouth through a subcutaneous tunnel. No immunosuppressive agent was administered. The results were assessed by visual inspection of the secretion, and histopathological examination of the transplanted SMG.

Results Technically, all surgical procedures were successful. Clear secretion flowed out of the duct as soon as blood supply of the transplanted submandibular gland was reestablished. The secretion of the gland lasted for 5 days. As expected, an acute rejection reaction occurred after surgery because no immunosuppressive agents were used. Secretion from the transplanted SMG ceased within 5 days.

Conclusions A model of SMG allotransplantation can be established in miniature swine. The technique of submandibular gland allotransplantation is feasible.

Chin Med J 2006; 119 (6):482-487

Xerophthalmia is a significant cause of blindness, not only because the precorneal tear film is essential for corneal transparency, but also because the dry eye develops keratitis with ulceration and opacification.[1] Since 1986, several researchers have reported microvascular autologous submandibular gland (SMG) transplantation for treatment of severe xerophthalmia.[2-14] The SMG is transferred to the temporal fossa, and end-to-end anastomosis performed between the external maxillary artery and the superficial temporal artery, and between the facial vein and the superficial temporal vein by using this technique. The secretory duct is transplanted to the upper lateral conjunctiva fornix. Gland secretions drain to the dry eye as a substitute for lacrimal fluid. Although clinical results have demonstrated that SMG autotransplantation is effective for severe xerophthalmia, there are still some problems that this technique can not solve. In some xerophthalmia patients, insufficient lacrimal secretion is accompanied by salivary gland hypofunction. For example, one study showed 11 of 26 patients (42%) recovering from toxic epidermal necrolysis exhibited a dry eye, and 7 of the 11 patients had decreased salivary flow.[15] If autotransplantation using the hypofunctional SMG had been performed in these patients, xerostomia would occur, and the transferred gland would not secrete the required amount of fluid. Another problem in autotransplantation is that the SMG will be lost if the transplantation fails. Failure to correct insufficient lacrimal gland secretion will result in loss of eyesight in severe xerophthalmia patients.

Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology, Beijing 100081, China (Ge XY, Yu GY, Cai ZG and Mao C)

Correspondence to: Dr. YU Guang-yan, Department of Oral and Maxillofacial Surgery, Peking University School of Stomatology, Beijing 100081, China (Tel: 86-10-62191099. Fax: 86-10-62174302. Email: gyyu@263.net)
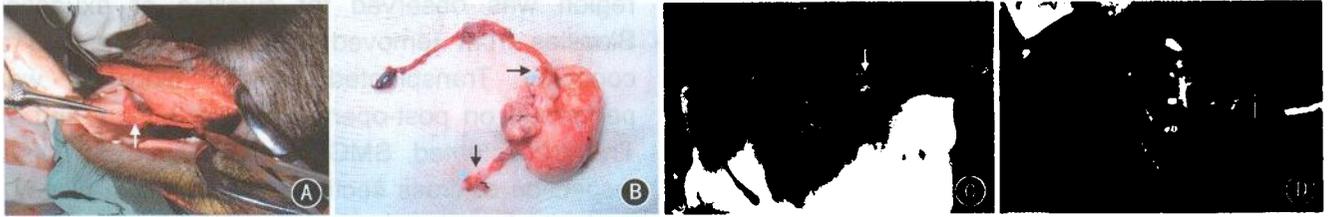
Fig. 1. Surgical technique of submandibular gland (SMG) allotransplantation in miniature swine. A: The isolated donor SMG duct in the mouth (↑). B: An excised donor SMG. The ends of the external maxillary artery (→) and external jugular vein (↓) are indicated. C: The donor gland after arterial (→) and venous (↓) anastomoses. D: The orifice of the duct after suturing to the mucous membrane of the vestibule of the mouth (→).

SMG allotransplantation is a potential treatment for severe xerophthalmia when the patient's SMG is hypofunctional or lost. If allotransplantation of SMG is feasible, this approach might be used to prevent blindness.

To evaluate the technical feasibility of SMG allotransplantation for treatment of severe xerophthalmia, it is essential to establish a suitable animal model. Miniature swine are comparable to humans in many aspects of anatomy and immunity, leading to their extensive use in organ allotransplantation research. To our knowledge, there is no miniature swine model for SMG allotransplantation. As for other types of organ transplantation, re-establishment of the blood supply is the primary requisition in SMG transplantation. In addition, SMG secretions drain via a duct, thus a suitable site for the duct is also important in an animal model.

METHODS

Experimental animals

Twelve miniature swine, provided by the Chinese Experimental Miniature Swine Stock Farm were used, they were 3—4 months old and weighed 10—20 kg. The care procedures were in accordance with the Principles of Laboratory Animal Administration organized by the Ministry of Science and Technology of the People's Republic of China. Two non-brood miniature swine were selected as donor and recipient. Donor and recipient were matched for weight and blood group (all type O). Males and females were randomized and tissue matching was not performed. Six SMG transplants were performed.

Anesthesia

The animals were fasted for 12 hours and drank water ad libitum before surgery. Each miniature swine was anesthetized with an intramuscular injection of 10 mg diazepam and 30 mg/kg ketamine hydrochloride. Ketamine hydrochloride was injected once every 40—50 minutes.

Donor operation

A submandibular incision was made and the SMG was dissected to identify its duct, artery and vein. The blood supply of the SMG was provided by the external maxillary artery and the facial vein. The external maxillary artery was traced as far as possible to its initial point, with the vein traced to the external jugular vein, and the duct dissected as far as possible towards the oral cavity.

The orifice of the duct was incised with a cuff of mucous membrane from the floor of the mouth. To facilitate the operation, a 2-cm incision was made at the floor of the mouth. The duct was dissected free and removed from the submandibular incision (Fig. 1A). The gland was left on its arterial and venous pedicles. After the recipient area was prepared, the external maxillary artery and external jugular vein were severed, and their proximal ends ligated. The SMG was removed from the donor (Fig. 1B), placed in a tray of cold (4°C) normal saline and irrigated through its artery with 100 ml of a 4°C solution of saline containing 12500 U heparin and 10 ml 2% lidocaine until it became pale. The field of operation was irrigated and the incision was closed.

Recipient operation

Preparation of the recipient site

A submandibular incision was made on the same side as that made on the donor animal, and the SMG was dissected to the external maxillary artery and external jugular vein. The external maxillary artery and

duct were ligated and severed. The distal end of the external jugular vein was ligated and divided after placing a vascular clamp on the proximal end. The SMG was removed and some of the tissue preserved in 10% formalin as a control. The lingual artery was identified and dissected free from surrounding tissue in the carotid triangle. In this region, the distal end of the lingual artery was ligated and divided after placing a vascular clamp on the proximal end. The proximal ends of the lingual artery and the external jugular vein were then trimmed for anastomosis.

Implantation of the SMG

Under an operating microscope, a 10-0 nylon suture was used to anastomose the artery of the gland to the lingual artery, and the vein of the gland to the external jugular vein using microvascular techniques. After anastomosis was completed, the vascular clamps were released from the artery and then the vein. After blood flow was re-established, the gland turned red in color and a clear secretion was observed flowing from the orifice of the duct (Fig. 1C). An incision was made in the mucous membrane in the vestibule of the mouth. A patch of mucous membrane was excised that matched the cuff of mucous membrane from the donor SMG. The secretory duct was drawn out through a subcutaneous tunnel and its end fixed to the incision using 6-0 nylon suture (Fig. 1D). The SMG was kept cool (about 4°C) by irrigating its surface during anastomosis. Care was taken to avoid twisting the blood vessels and the duct. The gland was fixed to the surrounding tissues to assist in aligning blood vessels and the secretory duct into suitable positions. The wound was irrigated with normal saline and closed with interrupted nylon sutures. A piece of rubber drainage strip was inserted into the wound to continuously drain fluid from the surgical site.

After transplantation, recipients were housed in separate cages in light-, temperature-, and airflow-controlled rooms and were fed with a liquid diet and water ad libitum for 7 days. After 24 hours, the drainage strip was removed. For infection prophylaxis, 80 000 units/day penicillin G was administered intramuscularly to all recipients for 7 days. Secretion from the transplanted gland was visually inspected and the orifice of the duct was irrigated daily to avoid obliteration. The operation

region was observed for swelling or exudation. Biopsies from removed recipient SMG served as controls. Transplanted gland biopsies were performed on post-operative days 3, 5, 7 and 10. The transplanted SMG was removed for visual inspection of cross section when the final biopsy is performed.

RESULTS

Technically, all six SMG surgical procedures were performed successfully. Surgery time for SMG allotransplantation varied from 4 to 5 hours. The mean warm ischemic time was 1−3 minutes and mean cold ischemic time was 30−40 minutes. All six recipient miniature swine recovered from anesthesia without difficulty and were able to eat and drink. All animals survived to the end of the experiment (the day of final biopsy) and no infection, swelling or exudation occurred. Secretions from the transplanted SMG decreased with time and ceased within 5 days.

Normal submandibular gland

The normal SMG removed from the recipient miniature swine was soft and flexible. The cross section showed that the glandular lobule was red and distinguishable. Histological examination showed that, as in humans, the miniature swine gland contained both mucous and serous elements. The majority of the acini were a mixture of mucous and serous cells and most of the remaining cells were serous acini (Fig. 2A). The excretory duct, striated duct and intercalated duct were similar to those in humans. Electron microscope examination showed that the ultramicrostructure of the miniature swine gland was also similar to that of humans. The apical cytoplasm was filled with secretory granules in serous cells, each surrounded by a unit membrane. In mucous cells, the secretory material was stored in droplets (Fig. 2B). Myoepithelial cells were also found at the periphery of the acini and in the intercalated ducts occupying the space between the basement membrane and basal plasma membrane of secretory cells.

Post-transplant histological examination
Gross appearance

On day 3 after surgery, visual inspection of the transplanted gland *in situ* revealed it to have a normal,
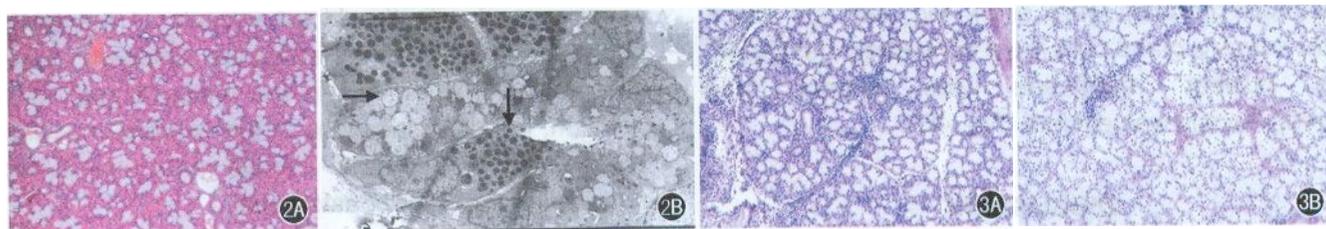
Fig. 2. Histological and ultrastructural appearance of a normal submandibular gland. **A:** Hematoxylin and eosin stain, original magnification ×10. **B:** Secretory granules (→) and mucous droplets (↓) in acinar cells; original magnification × 5000.

Fig. 3. Histological appearance of transplanted submandibular gland. **A:** Day 5 after surgery. **B:** Day 7 after surgery (Hematoxylin and eosin stains, original magnification ×10).

red color and be flexible. The cross section of the biopsy of the gland showed that the glandular lobule was distinguishable. On the 5th day after surgery, the gland was slightly swollen and felt hard. The color of the gland was deep red. The cross section showed hemorrhage and infarction. The glandular lobule was still distinguishable. On day 7 and 10 after surgery, the gland was smaller and felt hard. The cross section showed that the glandular lobule was vague and necrotic.

Appearance under light microscope

On day 3 after surgery, the gland structure was basically normal, and the border of gland lobule was clear. The parenchyma of the gland showed slight edema. There was mild dilatation and congestion of capillary vessels, and there were occasional lymphocytes inside and outside of these vessels.

On the 5th day after surgery, histologic examination showed infiltration of inflammatory cells into the parenchyma of the gland. The majority of inflammatory cells were lymphocytes, and the remainder were monocytes, plasmocytes and eosinophil granulocytes. The infiltrating inflammatory cells mainly appeared between the gland lobules and surrounding the ducts. Blood vessels were found to be dilated and congested. Serous and mucous cells were clear and regular, and the acini were intact (Fig. 3A).

On day 7 after surgery, a rejection reaction was more obvious. Inflammatory cells were diffusely present throughout the parenchyma of the gland, and the acini were impaired. Some acini were completely destroyed and only the contour was left. Thrombosis and necrosis was observed in blood vessels, and there were multiple hemorrhagic foci (Fig. 3B).
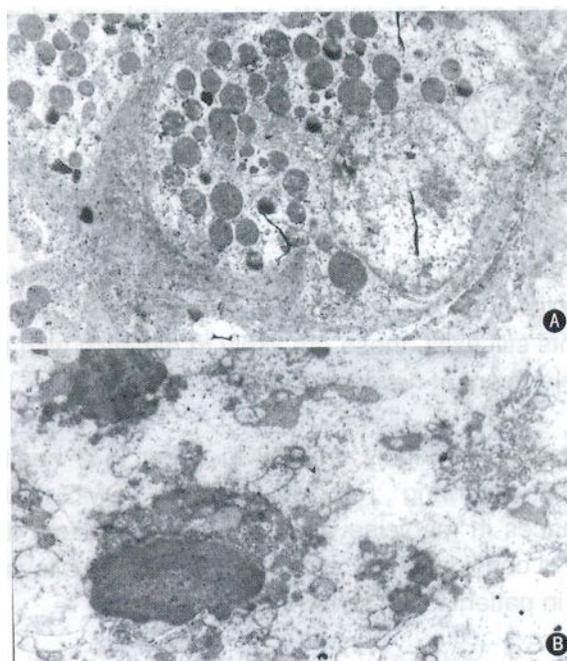

Fig. 4. Ultrastructural appearance of transplanted submandibular gland following electron microscopy (Original magnification ×5000). **A:** Day 3 after surgery. **B:** Day 7 after surgery.

On day 10 after surgery, the contour of the acinus was vague. The acinar cells showed complete necrosis and only nuclear fragments remained.

Ultrastructural appearance under electron microscopy

On days 3 and 5 after surgery, cell membrane structure was vague, and cell-cell junctions were impaired. The electron density of the cytoplasm in the transplanted SMG was lower than in normal SMG. The cell nucleus showed swelling, and the cell organs had dissolved, with mucous droplets merging in mucous cells. The electron density of secretory granules was low in serous cells, and fragments of

secretory granules were seen (Fig. 4A). On day 7 after surgery, gland cells were completely destroyed, the nuclear membrane was absent, and karyopyknosis was evident (Fig. 4B).

DISCUSSION

Transplantation medicine is the best example of the tremendous progress in medical practice that has been achieved in the last century. In earlier years, organ transplants were performed using organs essential for human life. In recent years, many new types of organ transplants and surgical procedures, and the highly potent immunosuppressive agents appeared gradually. Today, transplants are also performed to improve quality of life. For example, hand allotransplantation and free vascularized bone allotransplantation have achieved encouraging outcomes.[16-18] Opinions regarding organ transplantation have evolved over time. Although complications associated with immuno- suppressive agents still exist, these are likely to diminish with the development of new medicines.

Xerostomia is highly unlikely to occur in a healthy person following removal of one SMG. With advances in medicine and surgery, we believe it is time to consider SMG allotransplantation to alleviate pain in patients with severe xerophthalmia.

Revascularized SMG autotransplantation has been achieved in rats and rabbits.[1,3,19,20] Due to anatomical and physiological similarities with humans, miniature swine are a better model than rats and rabbits, and have been used extensively in organ transplantation research.[21] Establishment of an SMG allotransplantation model in miniature swine will provide the basis for clinical application of this procedure.

In autologous SMG transplantation, the SMG was transferred into the temporal region of the skull.[2-10] The vascular anastomoses were performed between the supplying vessels of the gland and the superficial temporal vessels. The SMG secretory duct was implanted into the conjunctival fornix. We have studied the anatomy of the miniature swine for SMG transplantation and found the temporal region was not suitable to be a recipient site because the superficial temporal vessels are too small to be anastomosed with the blood vessels of the SMG, and there are no other adequate blood-supplying vessels for anastomosis in the temporal region. In addition, the deep locations of the external maxillary artery and the external carotid artery were not suitable for vascular anastomoses.

The lingual artery passes deep into the stylohyoid and hyoglossus muscles, and superficial to the chondroglossus and genioglossus, and ascends along the genioglossus muscle to enter the substance of the tongue. It tapers from its origin and its caliber which is similar to the external maxillary artery when it passes deep into the hyoglossus muscle. It is relatively superficial in this region. In this study, it was convenient to use the lingual artery of the recipient as the blood-supplying artery.

Some patients develop obliteration of the SMG duct after autotransplantation, possibly due to surgical damage. In the present study, we did not severe the duct to avoid the possibility of the incision scar causing obliteration. Suturing the orifice of the duct to the floor of the mouth may cause damage to the many blood vessels in this region. To avoid this potential hemorrhage problem, we sutured the orifice of the duct to the mucous membrane of the vestibule of the mouth. This position is also convenient for observation of secretion.

Many tiny vein drainage branches of the SMG converge at the gland portal, and then join the facial vein. The facial vein joins the external jugular vein with the maxillary vein. The external maxillary artery and the secretory duct also pass by the SMG portal. Excessive dissection should be avoided near the gland portal in order to prevent damage to the blood vessels and the duct.

Since miniature swine are similar to humans in the histology of the SMG, they provide a satisfactory histological model for SMG allotransplantation research. To our knowledge, this is the first SMG allotransplantation model to be established in miniature swine. In the present study, the surgical technique of allotransplantation per se was successful, although acute rejection occurred later due to the absence of immunosuppressive agents. Further studies are needed to determine whether allografts can survive and perform adequate secretory functions over a long period in the

presence of safe immunosuppressive agents. If so, allotransplantation of the gland is probably a feasible treatment for severe xerophthalmia to avoid blindness.

REFERENCES

1. Kumar PA, MacLeod AM, O'Brien BM, Hickey MJ, Knight KR. Microvascular submandibular gland transfer for the management of xerophthalmia; an experimental study. Br J Plast Surg 1990; 43: 431-436.

2. Geerling G, Sieg P, Bastian GO, Laqua H. Transplantation of the autologous gland for most severe cases of keratoconjunctivitis sicca. Ophthalmology 1998; 105:327-335.

3. Kumar PA, Hickey CJ, Gurusinghe CJ, O'Brien BM. Long term results of submandibular gland transfer for the management of xerophthalmia. Br J Plast Surg 1991; 44: 506-508.

4. MacLeod A, Kumar PA, Hertess I, Newing R. Microvascular submandibular gland transfer; an alternative approach for total xerophthalmia. Br J Plast Surg 1990; 43: 437-439.

5. Murube-del-Castillo J. Transplantation of salivary gland to the lacrimal basin. Scand J Rheumatol Suppl 1986; 61: 264-267.

6. 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: 554-560.

7. Yu GY, Zhu ZH, Mao C, Cai ZG, Zou LH, Lü L. Microvascular autologous submandibular gland transfer in severe cases of keratoconjunctivitis sicca. Int J Oral Maxillofac Surg 2004; 33: 235-239.

8. Yu GY, Zhu ZH, Mao C, Cai ZG, Zou LH, Lü L. Microvascular submandibular gland transfer for severe keratoconjunctivitis sicca: operation key points, prevention and management of complications. Chin J Stomatol (Chin) 2002; 37: 353-355.

9. Zhu ZH, Yu GY, Mao C, Cai ZG, Zou LH, Lü L, et al. Treatment of xerophthalmia by transplantation of autologous vascularized submandibular gland. Natl Med J China (Chin) 2002; 82:244-246.

10. Jia GX, Wang YX, Lü L, Wang XK, Li ZL. Reconstructive lacrimal gland with free submandibular gland transfer for management of xerophthalmia. Chin J Ophthalmol (Chin) 1998; 34:388-390.

11. Geerling G, Honnicke K, Schroder C, Framme C, Sieg P, Lauer I, et al. Quality of salivary tears following autologous submandibular gland transplantation for severe dry eye. Graefes Arch Clin Exp Ophthalmol 2000; 238: 45-52.

12. Geerling G, Sieg P, Meyer C, Bastian GO, Laqua H. Transplantation of autologous submandibular glands in very severe keratoconjunctivitis sicca. 2 year outcome. Ophthalmologe 1998; 95: 257-265.

13. Lauer I, Sieg P, Bahre M, Richter E. Salivary gland scintigraphy using technetium-99m-pertechnetate after autotransplantation of the submandibular salivary gland in the correction of dry eye. Eur J Nucl Med 1998; 25: 128-131.

14. MacLeod AM, Robbins SP. Submandibular gland transfer in the correction of dry eye. Aust N Z J Ophthalmol 1992; 20: 99-103.

15. Binaghi M, Koso M, Roujeau JC, Coscas G. Ocular complications of Lyell's syndrome: recent concepts apropos of 26 cases. J Fr Ophtalmol 1985; 8: 239-243.

16. Doi K, Akino T, Shigetomi M, Muramatsu K, Kawai S. Vascularized bone allografts: review of current concepts. Microsurgery 1994; 15: 831-841.

17. Dubernard JM, Owen E, Herzberg G, Lanzetta M, Martin X, Kapila H, et al. Human hand allograft: report on first 6 months. Lancet 1999; 353: 1315-1320.

18. Hofmann GO, Kirschner MH, Buhren V, Land W. Allogenic vascularized transplantation of a human femoral diaphysis under cyclosporin A immunosuppression. Transpl Int 1995; 8: 418-419.

19. Spiegel JH, Zhang F, Levin DE, Singer MI, Buncke HJ. Microvascular transplantation of the rat submandibular gland. Plast Reconstr Surg 2000; 106: 1326-1335.

20. Spiegel JH, Deschler DG, Cheney ML. Microvascular transplantation and replantation of the rabbit submandibular gland. Arch Otolaryngol Head Neck Surg 2001; 127: 991-996.

21. Kenmochi T, Mullen Y, Miyamoto M, Stein E. Swine as an allotransplantation model. Vet Immunol Immunopathol 1994; 43:177-183.