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British Journal of Oral and Maxillofacial Surgery xxx (2011) xxx–xxx
BRITISH Journal of Oral and Maxillofacial Surgery
www.bjoms.com

An experimental study of the management of severe keratoconjunctivitis sicca with autologous reduced-sized submandibular gland transplantation

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Accepted 13 October 2011

Abstract

We have evaluated transplantation of reduced submandibular glands for the treatment of severe keratoconjunctivitis sicca. Thirty-four rabbits were allocated into three groups: dry eye (controls, $n = 10$), transplantation of whole submandibular glands ($n = 12$), and transplantation of reduced submandibular glands ($n = 12$). Outcome measures included the results of Schirmer's test and the Rose Bengal test, and histological examination of the cornea and the transplanted gland. Volume of tears significantly increased after transplantation of the whole gland, but did not change after transplantation of the reduced gland compared with dry eyes induced preoperatively. Neither transplantion group had keratoconjunctivitis sicca postoperatively. There were no histological abnormalities in the transplanted tissues. The results that the surgical technique of using reduced submandibular glands for transplantation was feasible, and that the secretion from the reduced gland was similar to that from a normal lacrimal gland. In conclusion, transplantation of a reduced submandibular glands is feasible in the treatment of keratoconjunctivitis sicca.

Keywords: Submandibular gland; Autologous transplantation; Keratoconjunctivitis sicca

Introduction

Dry eye syndrome, or keratoconjunctivitis sicca, results from inadequacy of the quality or quantity of tears produced. As a result, the cornea and conjunctival epithelium cannot maintain normal function. Clinically, most cases are associated with a reduction in the volume of tears, and patients experience a dry, uncomfortable sensation, with associated sensitivity to light and impaired vision. If the problem persists, as in severe cases of keratoconjunctivitis sicca, the corneal surface becomes ulcerated and eventually vision is lost.

Since 1986 many research workers have reported microvascular transplantation of autologous submandibular gland for the management of the patients with severe keratoconjunctivitis sicca.[1–5] The gland is transplanted to the temporal region of the skull, and Wharton's duct is transplanted to the upper lateral conjunctival fornix. The basal secretion of a transplanted and revascularised gland offers the potential for a permanent autologous substitution for the production of tears. In successful cases the symptoms of dry eye disappear, with obvious relief of discomfort. Patients are eventually able to stop using artificial tears, and visual acuity improves.

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0266-4356/$ – see front matter © 2011 Published by Elsevier Ltd on behalf of The British Association of Oral and Maxillofacial Surgeons.
doi:10.1016/j.bjoms.2011.10.004

Please cite this article in press as: Ge X-y, et al. An experimental study of the management of severe keratoconjunctivitis sicca with autologous reduced-sized submandibular gland transplantation. *Br J Oral Maxillofac Surg* (2011), doi:10.1016/j.bjoms.2011.10.004

The quantity of basal secretion of the submandibular gland is greater than that of the lacrimal gland under normal conditions. As a result, in cases of transplantation of the whole gland, some patients develop epiphora, particularly when exercising or in hot environments. Yu et al. reported epiphora in 8 of a series of 38 transplantations of whole glands.[5] The routine management of epiphora is to excise part of the transplanted gland, which necessitates a further operation. Yu et al. suggested that one third of the basal secretion of the submandibular gland is sufficient to keep the eye moist.[5,6]

In hepatic transplantation there is a technique known as reduced liver transplantation. We hypothesised that a similar technique for transplantation of the submandibular gland, with initial reduced transplantation, could produce sufficient secretion of tears, yet at the same time avoid epiphora and the need for further operation.

As the submandibular gland is clearly much smaller than the liver, we were anxious about whether the blood vessels or Wharton's duct would be damaged if a section of the gland was cut off before transplantation. The reduction in the size of the gland may have reduced secretion to a suboptimal level, which would fail to treat the original dry eye problem adequately. A further concern was whether Wharton's duct might be obliterated. There was also the technical issue of which part of the gland, and how much, was needed.

In this study we have assessed the feasibility and effectiveness of the technique of transplantation of the reduced submandibular gland using a rabbit dry eye model.

Material and methods

Animals

Thirty-four rabbits, weight 2.5–3 kg, were allocated into 3 groups. In the control group, 10 rabbits had a dry eye induced on one side; in the group that had the whole gland transplanted, 12 rabbits similarly had a dry eye induced, following which the gland was transplanted into the temporal region on the same side. In the experimental group (transplant of reduced gland), the procedure was similar to that for the whole gland except that the 12 rabbits had one-third of the gland removed and the remaining two-thirds transplanted. All procedures involving animals were in accordance with the Principles of Laboratory Animal Administration established by the Ministry of Science and Technology of the People's Republic of China. All measures were taken to minimise any pain or discomfort.

Surgical technique

Dry eye model

Three per cent pentobarbital sodium 1 ml/kg was injected into the marginal veins of the ear. The anaesthetised rabbit was then placed on the operating table, the field of operation was disinfected and draped, and a curved incision was made along the infraorbital border and the exterior margin of the orbital cavity. After incision of the skin and subcutaneous tissue, dissection was continued to the orbital border, which was cut to expose the lacrimal gland. This was isolated upwards and outwards, and then excised. In the root side of the third eyelid, Harder's gland was isolated and removed. Finally, the nictitating membrane (third eyelid) was cut off at the base. Cotton swabs dipped in trichloroacetic acid were smeared on the palpebral conjunctiva in the upper and lower fornix for about 3–5 s. The conjunctival tissue samples were then washed with physiological saline.

Transplantation of the reduced submandibular gland

The gland was transplanted once the dry eye model had been established. The animal was placed on the side opposite to the dry eye, and a preauricular incision made on the same side as the earlier dry eye operation. After dissection of the skin and subcutaneous tissue, the superficial temporal vein and the end piece of the external carotid artery were dissected for blood supply. The animal was then placed supine, and a midline incision made in the submandibular region. The external jugular vein was visualised, and the submandibular gland venous return branch was dissected and protected for anastomosis. The gland itself was then dissected. The external jugular vein and the distal end of the external maxillary artery were dissected and cut off in the anterior aspect of the gland. After dissection had been extended as far as possible towards the floor of the mouth, Wharton's duct was cut. Similarly, after dissection as far proximally as possible, the proximal section of the external maxillary artery was ligated and cut. After isolation of as much of its length as possible, the external jugular vein was ligated and cut in the posterior pole of the gland. At this point the gland was removed together with the external maxillary artery, the external jugular vein, and Wharton's duct. Once it had been rinsed with physiological saline and bleeding had been arrested, the original incision was sutured.

One-third of the gland, the section that was furthest from the 'gland gate' where the blood vessels and Wharton's duct enter and exit, was cut and the remaining section clamped and ligated to avoid both bleeding and the formation of a salivary fistula (Fig. 1). The animal was placed in a lateral position. The superficial temporal vein and the end part of the external carotid artery were clamped, ligated, and cut in the recipient site, after which the vessels were prepared for later anastomosis. Using a 10× microscope, the proximal end of the external maxillary artery of the gland was anastomosed to the end part of the external carotid artery using a 10/0 suture. The superficial temporal vein was anastomosed to the external jugular vein with a 10/0 suture. Soon after the vessels had been anastomosed, we saw clear, bright saliva flowing through Wharton's duct. A tunnel was made by blunt dissection, through which Wharton's duct was dragged from the transplant site to the upper lateral conjunctival fornix. The end of the duct was incised lengthways for 1–2 mm to expand its orifice. The eyelid was turned open, and, using a

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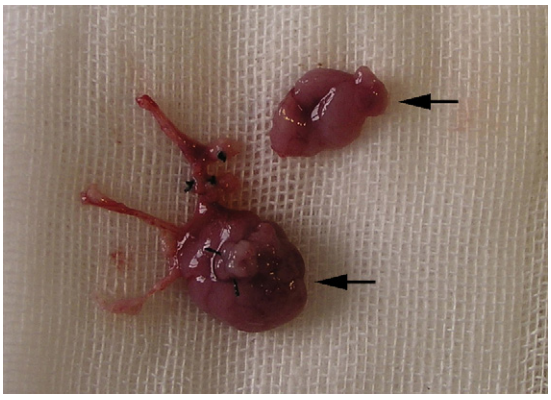

Fig. 1. The reduced submandibular gland (lower arrowhead) and the cut off part (upper arrowhead).

20× microscope, the end of the duct was fixed in the upper lateral conjunctival fold with an 11/0 suture. The original incision site was then rinsed with physiological saline, any residual bleeding stopped, and the site was sutured (Fig. 2).

Transplantation of the whole submandibular gland

The gland was removed in its entirety and transplanted. Otherwise, the procedure was similar to that of transplantation of the reduced gland.

Postoperative treatment

Tobramycin and dexamethasone eye drops were inserted 3 times daily for 3 days postoperatively for all animals.

Inspection and measurement

After the dry eye model procedure, the removed lacrimal and Harder's glands were fixed in formalin and embedded in paraffin to confirm their origin. All successful cases had a Schirmer's test and a 1% Rose Bengal staining test one day before, and 1–4 weeks, and 2 and 3 months after, the operation. All tests were done by the same investigator and under similar conditions.

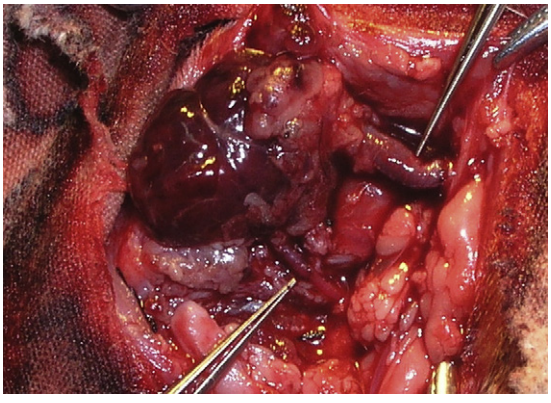

Fig. 2. The transplanted reduced submandibular gland after the vascular anastomosis. The forceps show the anastomosed vein (upper right) and artery (lower left).

Schirmer's test

A 5 mm × 35 mm Whatman 41 filter paper, one end of which was folded back 5 mm, was placed on the lower palpebral conjunctiva. The filter paper was removed after 5 min and the length of wetted paper measured. The test was performed 3 times for each examination and the mean value accepted as the result.

Rose Bengal test

One per cent Rose Bengal 1 ml was applied to the conjunctival sac, which was then rinsed with physiological saline. The resulting corneal staining was viewed under a white light. Lack of staining, punctiform staining, spot film staining, and sheet staining were classified as (−), (+), (++) and (+++), respectively.

Histopathological examination

All animals were killed 3 months after the operation. Each cornea and transplanted gland was fixed in formalin and embedded in paraffin. Pathological changes were observed using haematoxylin and eosin staining.

Statistical analysis

We used the Statistical Package for the Social Sciences SPSS version 13 (SPSS Inc., Chicago, IL, USA), and the significance of differences between groups were compared using analysis of variance (ANOVA). Tamhane's *T*2 test and the Student–Newman–Keuls test were used as appropriate.

Results

Four operations were unsuccessful because of faults in the technique, two in each of the transplantation groups, and these cases have therefore been excluded from analysis.

Schirmer's test

In the dry eye control group, homogeneity test of variance showed that equal variance was not assumed. The results of Schirmer's test before the operation were higher than afterwards ($P < 0.05$). There were no obvious differences in the measurements after the operation ($P > 0.05$).

In the group in which the whole gland was transplanted, the quantity of tears secreted after the operation was higher than before (Table 1). There were no differences between the quantity of tears produced before, and 1 week after, the operation ($P > 0.05$). The other postoperative measurements were significantly higher than those seen preoperatively ($P < 0.05$).

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Table 1
Production of tears before and after operation in the three groups. Data are mean (SD) mm.

Time	Dry eye alone ($n = 10$)	Whole gland transplant ($n = 10$)[a]	Reduced gland transplant ($n = 10$)[a]
Preoperatively	16.0 (5.72)	17.1 (4.93)	17.3 (3.74)
1 week	6.3 (2.16)	22.4 (6.36)	18.6 (3.5)
2 weeks	5.9 (1.97)	25.1 (6.79)	18.7 (5.27)
3 weeks	4.6 (1.26)	25.4 (7.37)	20.3 (4.62)
4 weeks	4.9 (1.6)	24.8 (7.24)	21.6 (5.87)
2 months	4.8 (1.55)	25.0 (7.01)	21.4 (6.82)
3 months	5.6 (2.07)	24.8 (7.04)	21.8 (7.11)

[a] 2 rabbits in each group were excluded.

There were no significant differences in results of Schirmer's test between any of the postoperative assessments ($P > 0.05$).

In the group in which the reduced gland had been transplanted, there was no significant difference between preoperative and postoperative production of tears ($P > 0.05$) (Table 1).

Rose Bengal test

In the dry eye control group, the 1% Rose Bengal test showed no staining (−) before the operation. One week postoperatively the cornea in some cases showed punctiform staining (+), and as time progressed, the extent of the staining gradually increased. After four weeks, all cases showed sheet staining (+++).

One case in each of the transplantation groups had punctiform staining (+), but the staining had disappeared by the second week. In all other rabbits in the transplantation groups there was no staining preoperatively or throughout the entire follow-up period (Fig. 3).

Histopathological examination

In the dry eye control group, the number of layers of corneal epithelial cells was increased. The appearance of the epithelial cells on the surface was irregular, and an inflammatory cell infiltrate was present in the insubstantial propria layer.

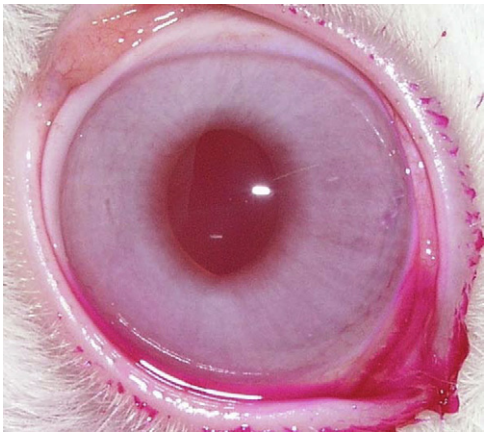

Fig. 3. Results of the Rose Bengal test in the group after transplantation of a reduced submandibular gland showing no staining (−).

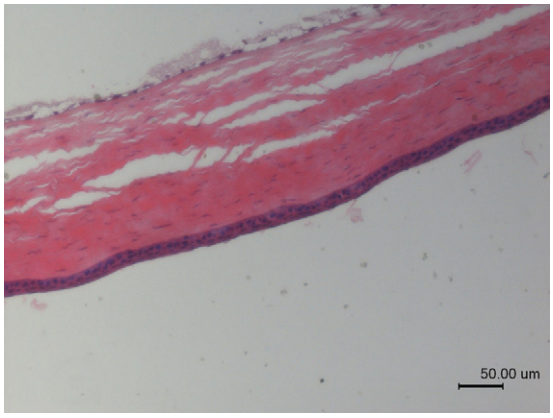

Fig. 4. No abnormal changes were found in the cornea after transplantation of a reduced submandibular gland at 3 months (haematoxylin and eosin; original magnification 10 × ???).

There was no abnormal corneal epithelium and no abnormal histological changes in either the whole or reduced glands (Figs. 4 and 5).

Discussion

Autotransplantation of the submandibular gland provides long-term pain relief and reduces the need for frequent instillation of lubricants.[7] However, the volume of tears required

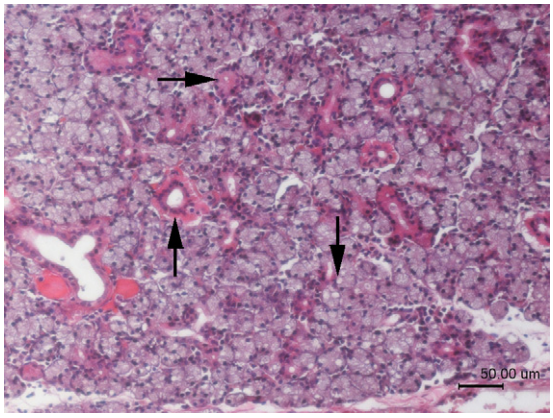

Fig. 5. Histological evidence of survival of transplanted reduced submandibular gland: serous cell (→); mucous cell (↓); duct (↑) (haematoxylin and eosin; original magnification 10 × ???).

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to ensure a moist eye surface is significantly less than the secretory volume of the normal gland. In some cases, oversecretion of the transplanted gland can cause epiphora, which is even more of a problem in the patients who had the operation for obstruction of the nasolacrimal duct to relieve the dry eye before autotransplantation of the gland. Regulation of glandular secretion can be managed only by reducing the size of the gland.[5] Further resection to achieve this may be necessary to reduce epiphora after transplantation of the entire gland.

Our microanatomic study showed a characteristic treelike structure of the vascular and duct system of the human submandibular gland.[8] It also possesses a portal structure similar to that in the liver where the blood vessels and ducts enter and exit. Our exploration of the anatomy of the gland in the rabbit during the preoperative period showed that it had a portal structure similar to that seen in humans. Apart from the portal site, no major vessels or ducts enter or exit the gland.

We hypothesised that transplantation of a reduced gland would not adversely affect blood flow or the flow of secretory fluid, and the results of the study confirm this. Initially, as in liver transplantation, we planned to conserve the main part of the gland, the blood vessels, and Wharton's duct, and to split the gland and transplant the section that contained the blood vessels and the duct. However, after dissection we discovered that the remaining blood vessels and the duct for transplantation were too thin for anastomosis if we conserved the part of the gland with the blood vessels and Wharton's duct, so we gave up the attempt.

When the whole gland was transplanted epiphora did occur in some cases, and what we saw in rabbits was similar to that seen in patients. Schirmer's test showed that there was no significant difference between preoperative and postoperative secretion of tears in the group in which the reduced gland was transplanted. The results suggest that if the reduction is adequate, secretion of tears in the group with the reduced gland is similar to that of a normal lacrimal gland, a satisfactory outcome can be achieved, and epiphora avoided.

The results of the Rose Bengal test and the histopathological examination suggested that transplantation of a reduced gland, like that of a whole gland, can be done without damage to the ocular surface. Patients with keratoconjunctivitis sicca, which is different from the surgically induced dry eye model in rabbits, often develop hypofunctional changes in the gland. The function of the gland to be transplanted therefore needs to be assessed clinically preoperatively. Scintigraphy with ^{99m}Tc pertechnetate may help to define the gland preoperatively.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 30730102 and 81070847), the National Supporting Program for Science and Technology (No. 2007BAI18B11), the Capital Medical Development Foundation (No. 2005-2009), and Science Foundation of Peking University School and Hospital of Stomatology.

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