Treatment of Severe Keratoconjunctivitis Sicca by Parotid Duct Transposition after Tympanic Neurectomy in Rabbits

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PURPOSE. To investigate the feasibility of parotid duct transposition after tympanic neurectomy to treat severe keratoconjunctivitis sicca (KCS) in rabbits.

METHODS. Thirty rabbits were divided into three groups in experiment 1. One eye was operated on, and the contralateral eye served as the control. In the KCS group, the lacrimal gland, harderian gland, and nictitating membrane were removed. In the group with parotid duct transposition (DT), the parotid duct was transposed into the lower conjunctival fornix. In the group with parotid duct transposition after tympanic neurectomy (DTTN), the tympanic nerve was resected in addition to parotid duct transposition. Schirmer test was performed and density of corneal staining was determined monthly after surgery, and goblet cell density was measured at postoperative month 3. In experiment 2, the tympanic nerve was resected on one side in 12 rabbits. Both sides of the parotid gland were resected for histopathology at intervals of 2 months to 1 year after surgery.

RESULTS. Tear secretion from operated eyes at rest increased significantly after surgery in the treatment groups compared with the KCS group. Tear secretion from operated eyes after chewing was significantly lower in the DTTN than in the DT group. The corneal staining scores were higher in the operated than in the control eyes of the three groups, without significant difference among the operated eyes. Parotid gland atrophy on the operated side occurred at postoperative month 4 and recovered to normal 1 year after surgery.

CONCLUSIONS. Parotid duct transposition after tympanic neurectomy could effectively reduce gustatory epiphora but may be insufficient to promote ocular surface health. (Invest Ophthalmol Vis Sci. 2011;52:6964–6970) DOI:10.1167/iovs.10-6459

Keratoconjunctivitis sicca (KCS), dry eye syndrome, is a relatively common disease characterized by reduced or no tears. Severe KCS leads to corneal ulceration, opacification, or even blindness. Traditional therapies for KCS are palliative and include artificial tear substitutes and occlusion of tear drainage to replace or conserve the patient’s tears. These treatment modalities give satisfactory results in mild and moderate cases but are not effective in severe cases.1

In the early 1950s, a treatment for KCS involved transposing the parotid duct into the lateral conjunctival fornix for a natural and continuous source of tears.2 However, this procedure could cause ectropion, epiphora, gustatory secretion, and traumatic keratitis because of the constant wiping of the eyes to remove excess secretion,3 and it was abandoned.

Microvascular autologous transplantation of submandibular glands with implantation of Wharton’s duct into the upper conjunctival fornix to treat severe KCS was introduced by Munne-del-Castillo4 in 1986 and was tested by several groups.1,5–8 This procedure could offer a permanent autologous source of tears with the basal secretion of a transplanted revascularized but denervated submandibular gland and so overcome gustatory epiphora. However, the surgical procedure was complicated, and a perfect microsurgical technique was needed.

To overcome this problem, we hypothesized that gustatory epiphora after transposition of the parotid duct could be avoided if the parotid gland was denervated. Most of the parasympathetic nerve supply to the parotid gland is via the tympanic nerve. Parotid secretion on gustatory stimulation could decrease if the tympanic nerve were resected. Tympanic neurectomy was first used in 1962 by Golding-Wood.9 In this surgery, the parasympathetic nerve supply of the parotid gland is interrupted by dissecting the tympanic nerve on the promontory of the middle ear. Tympanic neurectomy has been used successfully to treat patients with drooling,10 parotid fistula,11 Frey’s syndrome,12 and chronic parotitis.13

We aimed to investigate the feasibility of parotid duct transposition after tympanic neurectomy to treat severe KCS in rabbits. We hoped to create a new therapeutic approach for severe KCS and provide the experimental basis for its clinical application.

MATERIALS AND METHODS

The study involved 30 Japanese albino rabbits of both sexes weighing 2.0 to 3.0 kg. The rabbits were randomly divided into three groups (n = 10 each). All rabbits were bred by Peking University Laboratory Animal Center and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animals were anesthetized with intravenous pentobarbital sodium (30 mg/kg). Only one eye was operated on in each animal. The contralateral eye was used as the control. All measurements and assays of eyes were performed in a masked fashion.

The KCS model was created in all rabbits by surgical removal of the lacrimal gland and the harderian gland and nictitating membrane, as described elsewhere.14 In the parotid duct transposition (DT) group, the ipsilateral parotid duct was transposed into the lower conjunctival fornix. In the group with parotid duct transposition after tympanic...
neurectomy (DTTN), in addition to parotid duct transposition, the tympanic nerve was resected.

A second experiment was designed to evaluate the long-term histopathologic changes and secretion function of the denervated parotid gland. Twelve Japanese albino rabbits of both sexes weighing 2.0 to 3.0 kg were anesthetized with intravenous pentobarbital sodium (30 mg/kg). The tympanic nerve was resected on one side in each animal. The other side served as the control. Two rabbits in experiment 2 were killed at each time point at intervals of 2 months to 1 year after tympanic neurectomy.

**Tympanic Neurectomy**

A curve-shaped incision was made on the posterior and inferior limbus of the ear (Fig. 1A). Skin was incised and the cartilaginous auditory canal was exposed (Fig. 1B). The cartilaginous auditory canal was incised from the tragus to the origin of the bony auditory canal (Fig. 1C). Under the operating microscope, the mucosa was freed from the floor of the bony auditory canal. The middle ear was opened by inferior and posterior mobilization of the drum. In the direction of the hypotympanum, the tympanic nerve passes toward the promontory, where it usually lies in a small mucosally covered bony groove (Fig. 1D). Tympanic neurectomy was performed by subluxing the tympanic nerve from its bony groove and removing a segment of the nerve as far as possible. To coagulate any remnants of neural or mucosal tissue that had escaped instrument destruction, the promontory was swabbed with 50% trichloroacetic acid until the mucosa on the promontory turns black. Arrow: the chorda tympani nerve. (F) The skin is sutured.

**Parotid Duct Transposition**

A horizontal skin incision was made in the anterior region of the cheek of rabbits (Fig. 2A). The parotid duct was located and transected in the distal end of the duct. Then, the parotid duct was freed up to the proximal end of the duct (Fig. 2B). By blunt dissection, a tunnel to the lower conjunctival fornix was made, and the duct was passed through it (Fig. 2C). Under the surgical microscope, the distal end of the duct was sutured to the margins of the conjunctival incision with 8-0 nylon sutures (Fig. 2D). The skin wound was then closed (Fig. 2E).

**Postoperative Treatment**

In total, 400,000 U penicillin (North China Pharmaceuticals, Hebei, China) was administered intramuscularly, twice daily for 3 days, and eye drops with 0.3% tobramycin and 0.1% dexamethasone were given three times daily for 5 days. All animals in experiment 1 were killed by intravenous pentobarbital sodium overdose at postoperative month 3.
Schirmer Test

Strips of filter paper 5 mm in width were folded at the 5-mm notch and placed into the lower medial third of the conjunctival fornix of both eyes for 5 minutes without anesthesia before and after the rabbits chewed food pellets. The rabbits were strapped down and fixed in the wooden box, and the eyes were closed with hands after the strips were inserted, to avoid their scratching their eyes to remove the test strips. The length of moisture on the paper was measured. The test was repeated three times, and the mean value was obtained. The test was performed before surgery and monthly for 3 months after surgery.

Assessment of Amylase Activity of Tears

The tear samples were collected by a microcapillary tube placed near the lacrimal lacus on the operated eyes of the DT and DTTN groups. Normal tear samples were obtained by stimulating the conjunctiva with a microcapillary tube before surgery. The tear specimens were collected monthly after surgery after the rabbits chewed food pellets. All the tear samples were collected between 9 and 11 AM and immediately stored in 0.5-mL vials (Eppendorf, Fremont, CA) at –80°C until analysis. Amylase activity of the tear was measured using ethylidene-4-nitrophenyl-D-maltoheptaoside (Leadman Group Co., Ltd., Beijing, China) as a substrate. Bichromatic readings were made at 405 and 546 nm on an automatic biochemical analyzer (LX20; Beckman, Fullerton, CA).

Fluorescein Test

The eyes of all animals were checked monthly after surgery for 3 months with instillation of 1 drop of 1% fluorescein solution. Conjunctival staining was assessed by trained ophthalmologist (YJ). The modified scoring system was used according to the National Eye Institute/Industry Workshop on Clinical Trials for Dry Eyes.15 Conjunctival fluorescein staining was graded on a scale from 0 to 3: 0, no staining; 1, mild staining with a few disseminated stains; 2, moderate staining with a severity between grades 1 and 3; or 3, severe staining with confluent stains.

Rose Bengal Test

With rabbits under surface anesthesia, both eyes were checked after instillation of 1 drop of 1% solution of rose bengal on day 2 after each fluorescein staining. The scoring method was the same as that used for fluorescein staining.

Measurement of Goblet Cell Density

Bulbar conjunctival biopsy was performed on both eyes after animals in experiment 1 were killed at postoperative month 3. The conjunctival goblet cell density depends on the topographic location within the conjunctival field.16 Therefore, we obtained conjunctival biopsies superior to the cornea between the muscle rectus lateralis oculi and medialis oculi. Specimens were processed by routine techniques. The sections (6 μm) perpendicular to the conjunctival surface were rehydrated and stained with periodic acid Schiff (PAS). Conjunctival goblet cells were counted in five sections at ×40 magnification under an optical microscope.17 The mean value was recorded as the number of goblet cells per field.

Histopathologic Examination

All the rabbits for the histopathologic examination in experiment 1 were killed at postoperative month 3. The parotid gland and bulbar conjunctival and corneal biopsies were taken from the operated and control sides. Tissue samples were processed by routine techniques. The sections were stained with hematoxylin and eosin and observed under an optical microscope.

In experiment 2, two rabbits were killed at each time point at intervals of 2 months to 1 year after tympanic neurectomy. Both sides of the parotid gland were removed from each animal. Half the tissue samples were fixed in formalin and stained with hematoxylin and eosin (H&E) for routine histopathologic study. The other half of the sample were cut into small cubes and fixed in 2.5% glutaraldehyde with phosphate-buffered saline (0.1 M; pH 7.2–7.4) for 2 hours, postfixed in 0.1% OsO4 in the same buffered solution for 1 hour, and then dehydrated and embedded in epoxy resins. Ultrathin sections were counterstained with uranyl acetate and lead citrate and observed under a transmission electron microscope (400S; Philips, Eindhoven, The Netherlands).

Statistical Analysis

Data are presented as the mean ± SD. The tear secretion at rest in each group was analyzed before and after surgery by univariate ANOVA. The tear secretion in the DT and DTTN groups was analyzed, before and after chewing, by paired Student’s t-test each month after surgery. The tear secretion of the DT and DTTN groups after chewing was compared by independent-samples t-test at each time point after surgery. Amylase activity in the DT and DTTN groups was compared before surgery and after surgery by univariate ANOVA. The Kruskal-Wallis test was used to compare corneal staining in operated and control eyes. One-way ANOVA was used to compare goblet cell density in operated and control eyes. Multiple comparison was performed by Bonferroni tests. P < 0.05 was considered statistically significant (SPSS ver. 11.5; SPSS Inc., Chicago, IL).

RESULTS

Tear Secretion at Rest

For the KCS alone group, tear secretion from the operated eyes was significantly lower at all postoperative time points than before surgery (P = 0.000), indicating that the KCS model was established successfully.

After parotid duct transposition, tear secretion from the operated eyes increased significantly at all postoperative time points in both groups, with or without tympanic neurectomy, compared with the KCS group (P = 0.000; Fig. 3A).

Tear secretion from the operated eyes in the DTTN group was similar compared with that in the DT group at postoperative months 1 and 2, but was higher in the DT group at postoperative month 3 than in the DT group (DTTN group versus DT group at postoperative month 3, P = 0.037).

Tear Secretion after Chewing

For the DT group, tear secretion from the operated eyes significantly increased after chewing than at rest at postoperative months 1 to 3 (P = 0.001, P = 0.001; and P = 0.015 respectively; Fig. 3B).

Furthermore, tear secretion from the operated eyes after chewing was significantly lower in the DTTN group than in the DT group at all postoperative time points (P = 0.000; Fig. 3B), suggesting that the gustatory reflex was effectively controlled after tympanic neurectomy.

Amylase Activity of Tears

For the DT and DTTN groups, the amylase activity of tears in the operated eyes significantly increased at all postoperative time points compared with before surgery (P = 0.000; Table 1). The amylase activity of tears in the operated eyes was significantly higher only at postoperative month 3 in the DTTN group than in the DT group (P = 0.012).

Ocular Surface Changes

At monthly examination during the first 3 months after surgery, slit lamp examination showed abnormal fluorescein and rose bengal staining of the cornea in the operated eyes of the three groups (Fig. 4). Compared with the mean scores for contralateral control eyes, the mean corneal fluorescein staining scores
and the mean rose bengal staining scores in the operated eyes increased significantly in the three groups at all postoperative time points \( (P = 0.000) \), with no significant difference in the operated eyes between the three groups. Meanwhile, the extent of the corneal staining did not change with time in the three groups.

Goblet cell density was significantly lower in the operated eyes in the KCS group than in the DTTN group \( (P = 0.010) \); and was maintained after chewing food pellets \( (P < 0.01) \). However, the operated eyes of the two groups did not differ significantly in goblet cell density \( (P = 1.000) \).

### DISCUSSION

During the 1950s and 1960s, severe KCS was treated by transferring the parotid duct into the conjunctival sac.\(^{18-20}\) However, patients could not endure the gustatory epiphora, and this technique was discarded. To overcome this problem, several researchers tried to reduce the secretion of the operated parotid gland, but they failed to control the gustatory epiphora successfully without serious complications.\(^{2,21}\) Keegan et al.\(^{22}\) reported that intraglandular and periglandular injection of botulinum toxin might be useful to treat hyperlacrimation secondary to “crocodile tearing” or submandibular gland transplantation, but it was temporary. The saliva secretion from the parotid gland is predominantly regulated by the tympanic nerve. Since 1962, tympanic neuroectomy has been successfully applied to reduce saliva secretion of the parotid gland in treating drooling, parotid fistula, Frey’s syndrome, and chronic parotitis.\(^{25-26}\) It is a relatively mature technique for the otolaryngologists without serious complications.\(^{13}\) We hypothesized that gustatory epiphora with parotid duct transfer to treat KCS might be controlled by resecting the tympanic nerve. Therefore, we designed a protocol of transferring the parotid duct into the inferior conjunctival fornix after tympanic neuroectomy to treat severe KCS in rabbits. Our results indicated that

**TABLE 1. Comparison of Pre- and Postoperative Amylase Activity of Tears in the Surgically Altered Eyes in the DT and DTTN Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Preoperative</th>
<th>Month 1</th>
<th>Month 2</th>
<th>Month 3</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>82 ± 54</td>
<td>44.098 ± 9.888*</td>
<td>40.060 ± 2.263*</td>
<td>28.505 ± 13.092*</td>
<td>0.000</td>
</tr>
<tr>
<td>DTTN</td>
<td>48 ± 79</td>
<td>69.025 ± 54.072†</td>
<td>53.950 ± 24.285†</td>
<td>57.225 ± 24.975†</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Data were expressed as mean IU/L ± SD. \( n = 10 \) at each time point.

* \( P < 0.01 \) compared with before surgery in the DT group.

† \( P < 0.01 \) compared with before surgery in the DTTN group.

### Histopathology

A large quantity of basophilic secretion granules were observed in normal acinar cells of the parotid gland. The DT group showed many secretion granules in acinar cells of the parotid gland on the operated side at postoperative month 3, but the secretion granules decreased in the DTTN group. Neither control nor operated eyes of all three groups showed inflammatory pathologic changes of cornea and conjunctiva.

The 12 rabbits killed for histologic examination showed many secretion granules in normal acinar cells of the parotid gland and some fatty tissue in the lobes of the parotid gland (Figs. 7A, 7B, 8A). The secretion granules decreased in acinar cells of the parotid gland on the operated side at postoperative month 4 (Figs. 7C, 8B). Fatty tissue had infiltrated in the lobes of the parotid gland on the operated side at postoperative month 6 (Fig. 7D). The histopathologic changes of the parotid gland on the operated side recovered to normal 1 year after surgery, the fatty tissue in the lobes of the parotid gland decreased (Fig. 7E), and the secretion granules increased in acinar cells of the parotid gland (Fig. 7F, 8C), compared with that at postoperative months 4 and 6.
there was a significant decrease in the quantity of tear secretion in the operated eyes after chewing at all postoperative time points in the DTTN group compared with that in the DT group. The tear secretion from the operated eyes after chewing was much higher than secretion at rest in the DT group, while the tear secretion after chewing was similar to secretion at rest in the DTTN group. Therefore, gustatory epiphora after parotid duct transposition could be controlled by tympanic neurectomy.

Bernard demonstrated that a denervated salivary gland maintained a basal secretion after an initial lag period (see review by Kumar et al.27). Yu et al.1 reported that the secretion of the denervated autotransplanted submandibular gland, which was attributed to basal secretion from the transplanted gland, could lubricate the ocular structures in patients with severe KCS. Geerling et al.28 found that free submandibular autografts remained viable in the long term due to substantial survival of parasympathetic ganglia and sympathetic reinnervation in transplanted gland tissue. In the present study, tear secretion in the operated eyes with KCS alone significantly decreased within 3 months. The tear secretion from the operated eyes did not decrease after surgery in the DTTN group. Therefore, the denervated parotid gland did not lose the function of the secretion and maintained a basal secretion. In our study, tear secretion from the operated eyes did not significantly decrease at rest in the DTTN group compared with that in the DT group. As we know, saliva secretion is lower at rest in the parotid gland than in the submandibular gland, but significantly increases under stimulus. We speculated that the tympanic nerve predominantly regulates the saliva secretion of the parotid gland in response to stimulus, but not at rest. The mechanism of increased secretion at rest in the DTTN group at postoperative month 3 is not clear. Whether it was due to “paralytic” secretion needs further investigation. In the parotid gland on the operated side, the secretion granules
The saline solution was composed of 0.9% saline that had been filtered through a 0.22-μm pore filter (Nalgene), and the concentration of sodium was 140 mEq/L.

The concentration of potassium was 5 mEq/L, and the concentration of chloride was 140 mEq/L. The osmolality of the saline solution was measured using a Vapro 5500 osmometer (Vapro, World Precisions Instruments, Rye, NY) and was found to be 280 mOsm/L.

The saline solution was injected into the eye three times a day, with a total of 40–60 μl injected per eye per day. The saline solution was applied to the eye using a microsyringe (Hamilton, Reno, NV), and the volume of the saline solution used was recorded. The saline solution was discarded after it was used.

Results

The saline solution was well tolerated by all patients, and no adverse effects were reported. The saline solution was effective in treating dry eye, and the symptoms improved significantly after the treatment period. The patients reported an increase in the number of tears and a decrease in the frequency of tearing.

The saline solution was also effective in improving the corneal surface, as evidenced by the reduction in the corneal fluorescein staining score. The corneal fluorescein staining score was significantly lower in the eyes treated with the saline solution compared to the control eyes.

The saline solution was also effective in improving the quality of life of the patients. The visual analog scale (VAS) score, which measures the severity of dry eye symptoms, was significantly lower in the eyes treated with the saline solution compared to the control eyes.

Conclusion

The saline solution was effective in treating dry eye and improving the corneal surface and quality of life of the patients. The saline solution was well tolerated by all patients, and no adverse effects were reported. The saline solution is a safe and effective treatment option for dry eye.

Acknowledgments

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References

2.泪膜
3. dry eye.

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