Establishment of a Rabbit Model for Keratoconjunctivitis Sicca

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Purpose: To establish a rabbit model for keratoconjunctivitis sicca (KCS) to study autologous submandibular gland transfer for treating severe KCS.

Methods: In 2 groups of 10 rabbits, left eyes were operated and right eyes were controls. In the trichloroacetic acid-treated group, the lacrimal and harderian glands and nictitating membrane were removed surgically; the palpebral and bulbar conjunctiva were swabbed with 50% trichloroacetic acid. In the non-trichloroacetic acid-treated group, the lacrimal and harderian glands and nictitating membrane were surgically removed. The Schirmer test was performed preoperatively and 1, 2, 3, and 4 months postoperatively. Corneal densities of rose bengal and fluorescein staining were scored every month postoperatively. At 4 months, the cornea and bulbar conjunctiva were removed from operated and control eyes for histopathology. The upper bulbar conjunctiva was used to determine goblet cell density.

Results: Compared with preoperative conditions, tear secretion of operated eyes significantly decreased in both groups postoperatively, then gradually increased. Scores for corneal rose bengal and fluorescein staining were higher and conjunctival goblet cell density was lower in the operated eyes than in control right eyes in both groups, but no significant difference was found between the operated eyes of the two groups. Inflammatory histopathologic changes of the cornea and conjunctiva were not found in either of the eyes in the two groups.

Conclusions: A new rabbit model for KCS could be created by either of these methods. Experimental KCS with reduction of tear production was possible with surgical ablation of the lacrimal and harderian glands and nictitating membrane. It is unnecessary to apply trichloroacetic acid to burn the conjunctiva. Our modified incision better exposed the surgical field.

Key Words: keratoconjunctivitis sicca, goblet cells, ocular surface, tears

(*Cornea* 2011;30:1024–1029)

Received for publication August 24, 2009; revision received November 15, 2009; accepted November 21, 2009.

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Financially supported by National Natural Science Foundation of China (No 30730102), the Capital Medical Development Foundation (No 2005-2009), and National Supporting Program for Science and Technology (No 2007BAI18B11).

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In keratoconjunctivitis sicca (KCS), reduced tear secretion and altered tear composition damage the ocular surface. Severe KCS leads to corneal ulceration, opacification, or even blindness. Treatment of severe KCS is crucial, yet difficult. Since 1986, autologous submandibular gland transfer has been used to treat severe KCS.[1] Tear secretion increased because of a permanent autologous substitution of tears using the basal secretion of a transplanted and revascularized submandibular gland. In successful cases, the symptom of dry eye was relieved and patients could stop using artificial tears.[1] However, the transplanted submandibular gland is denervated, and its secretion mechanism is changed. The secretion of the transplanted gland is very low in the early stage of transplantation, which may result in obstruction of the duct and even failure of the operation.[1] Animal studies on the mechanism and regulation of secretion of transplanted submandibular gland are crucial. Establishment of a successful animal model of dry eye is necessary.

Numerous animal models of KCS, including rabbit,[2–5] dog,[6] rat,[7] and mouse,[8] have been developed. Rabbit is one of the most commonly used animal models in the experiments because of ease of feeding, low costs, and definite anatomy. However, no report of any successful rabbit model for KCS has appeared in the literature, particularly for studying autologous submandibular gland transfer in treating severe KCS.

In the past, Gilbard and Rossi[9] introduced a rabbit model of KCS by closing the lacrimal gland excretory duct and removing the nictitating membrane and harderian gland. However, tear secretion did not decrease in their study. We found that this operation was difficult to perform because excretory ducts of the main lacrimal gland were not always obstructed throughout the experiment. Recently, some authors described a rabbit model of KCS in which they removed the lacrimal gland through a lateral orbital incision or a transconjunctival approach.[10,11] The disadvantages of this procedure were the poor exposure and incomplete ablation of lacrimal glands because of the limited surgical field.

The aim of our study was to create a new rabbit model of KCS by modifying those previously reported methods. We hoped that the new model could be used to study autologous submandibular gland transfer for the management of severe KCS.

MATERIALS AND METHODS

The study was conducted on 20 Japanese albino rabbits of both sexes weighing 2.0 to 2.5 kg. The rabbits were randomly divided into two groups of 10 each. All rabbits were bred by Peking University Laboratory Animal Center and

treated according to the China Laboratory Animal Welfare Law. Only the left eye was the operated eye in each animal. The right eye was used as the control. All measurements and assays were performed on all eyes in a masked fashion.

In group 1, the non-trichloroacetic acid-treated group, rabbits underwent surgical removal of the left lacrimal and harderian glands and the nictitating membrane. In group 2, the trichloroacetic acid-treated group, in addition to ablation of the left lacrimal and harderian glands and the nictitating membrane, the palpebral and bulbar conjunctiva were swabbed with 50% trichloroacetic acid, which could destroy the goblet cells of the conjunctiva with the aim to decrease the secretion of mucins in the mucous layer of the tear film and induce inflammation of the conjunctiva.

Operative Technique

All animals were anesthetized with intravenous sodium pentobarbital (30 mg/kg). In the non-trichloroacetic acid-treated group, a curve-shaped incision was made on the lateral and inferior orbital rims (Fig. 1A). The upper and lower eyelids were sutured to prevent dryness of the ocular surface during surgery. Skin was cut and the tarsus exposed. Dissection was done superficially to the tarsus until the orbital rim was seen. The orbital septum was transected along the orbital rim so that the lacrimal gland could be identified and dissected from its supporting tissues. The infraorbital part, temporal lobe, and intraorbital part of the lacrimal glands were removed (Figs. 1B–D). The harderian gland was separated and ablated anterior to the infraorbital lacrimal gland and deep to orbit (Fig. 1E). Gelatin sponge was tamponaded in the orbit for hemostasis. Wound closure was performed by apposing the orbital fascia with 4-0 nonabsorbable sutures, and the skin was sutured with 5-0 nonabsorbable sutures. The nictitating membrane was removed at the base with curved scissors. In the trichloroacetic acid-treated group, in addition to ablation of the left lacrimal gland, harderian gland, and nictitating membrane, a cotton swab soaked with 50% trichloroacetic acid, which was diluted in distilled water, was applied on palpebral conjunctiva and bulbar conjunctiva 3 to 4 mm lateral to corneal limbus for 3 minutes (Fig. 1F). When blanching of the conjunctiva was observed, irrigation with 0.9% sodium chloride solution was performed. A total of 400,000 U penicillin (provided by North China Pharmaceuticals, Shijiazhuang, Hebei, China) was administered intramuscularly, twice daily for 3 days. Eye drops with 0.3% tobramycin and 0.1% dexamethasone were given three times daily for 5 days postoperatively. All animals were killed by intravenous sodium pentobarbital overdose at 4 months after surgery.

Schirmer Test

This test was carried out using 5-mm strips of filter paper folded at the 5-mm notch and placed into the lower conjunctival fornix on both eyes for 5 minutes without anesthesia. The length of the moistened paper was measured. The test was done preoperatively and monthly for 4 months after surgery.

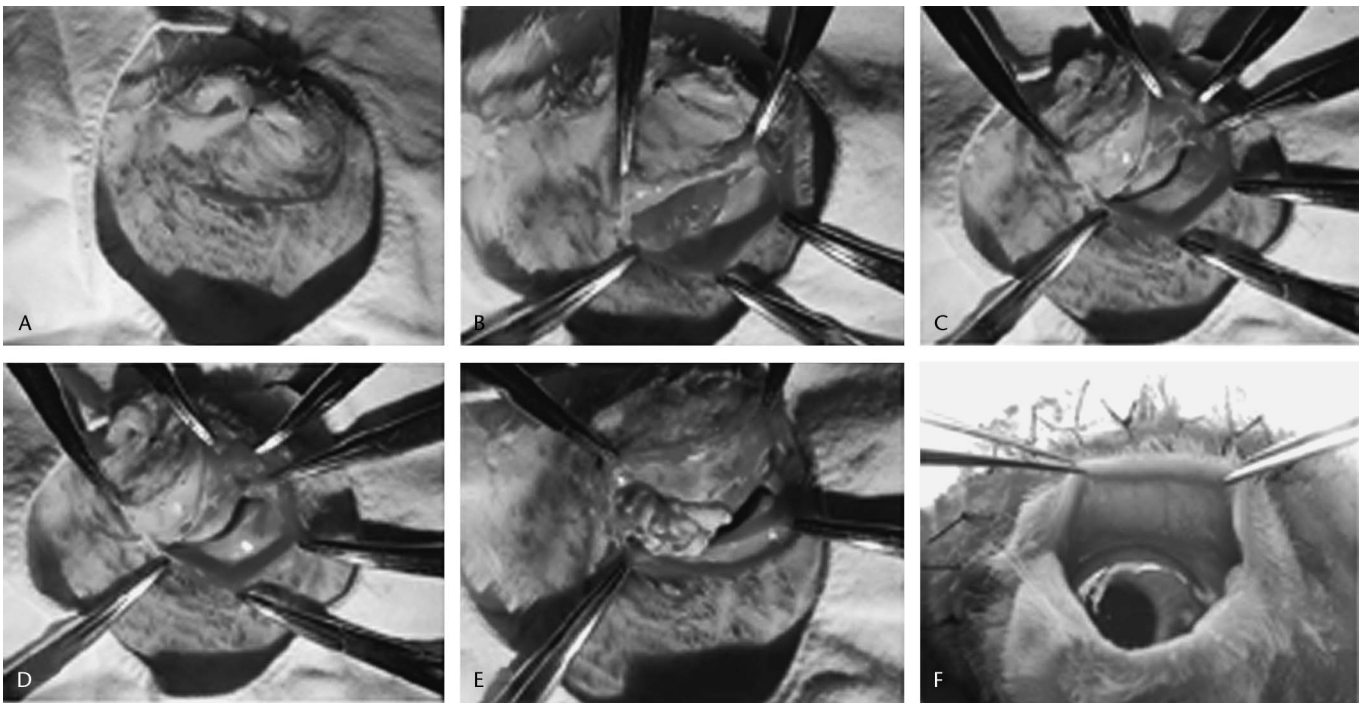

FIGURE 1. Surgical technique. A, Surgical approach; the upper and lower eyelids were sutured to prevent dryness of ocular surface during operation. B, Removal of infraorbital part of lacrimal glands. C, Removal of temporal lobe of lacrimal glands. D, Removal of intraorbital part of lacrimal glands. E, Removal of harderian glands. F, A cotton swab soaked with 50% trichloroacetic acid was applied to the palpebral conjunctiva and bulbar conjunctiva 3 to 4 mm lateral to the corneal limbus.

Fluorescein Test

The eyes of all animals were checked monthly with instillation of one drop of 1% fluorescein solution, beginning from the first month to the fourth month postoperatively. The assessment of the cornea staining was conducted by a trained ophthalmologist (Liang QF). The cornea was divided into four quadrants and the intensity of staining in each was scored, each section up to 3 points, so that a maximum score of 12 could be obtained.[12]

Rose Bengal Test

Both eyes of all animals were checked after instillation of one drop of 1% rose bengal solution under surface anesthesia on the second day after fluorescein staining. Rose bengal was absorbed by dead or dying cells. Positive staining occurred on the cornea with KCS. The scoring method was the same as that used in fluorescein staining.

Measurement of Goblet Cell Density

In the fourth month after surgery, all animals were killed by intravenous administration of sodium pentobarbital overdose. A bulbar conjunctival biopsy was then performed on both eyes. The conjunctival goblet cell density depends on the topographical location within the conjunctival field.[13] For this reason, we obtained a conjunctival specimen for biopsy superior to the cornea between the musculus rectus lateralis oculi and the musculus rectus medialis oculi. The specimen was fixed by 10% formaldehyde, dehydrated by alcohol and toluene, and embedded in paraffin. The sections (6 μm) perpendicular to the conjunctival surface were rehydrated and stained by periodic acid-Schiff. Five sections were selected, and the conjunctival goblet cells were counted at ×40 magnification under an optical microscope.[14] The average value was recorded as the number of the goblet cells per microscopic field.

Histopathologic Examination

The animals were killed at 4 months postoperatively. Bulbar conjunctival and corneal biopsies were taken from both the operated and control eyes. Tissue samples were then processed by using routine techniques. The sections were stained with hematoxylin and eosin and observed under an optical microscope.

Statistical Analysis

The tear production from each group of rabbits was analyzed preoperatively and postoperatively using a univariate analysis of variance. At each time point postoperatively, the data from the operated eyes in the two groups were compared using the independent sample *t* test. A Kruskal-Wallis test was used to compare the corneal-staining scores for both operated eyes and control eyes in the two groups at each time point postoperatively. The statistical significance of the goblet cell density was evaluated among both operated and control eyes using a one-way analysis of variance. Statistical analysis was performed on computer (SPSS ver.11.5; SPSS Inc., Chicago, IL). $P < 0.05$ was regarded as statistically significant.

RESULTS

Tear Secretion

In the non-trichloroacetic acid-treated group, a significant decrease in the quantity of tear secretion was found in the operated eyes at all time points after operation compared with that preoperatively (Fig. 2). A comparison of the quantity of tear secretion measured at 1, 2, 3, and 4 months after operation showed that tear secretion continued to increase up to the fourth month.

In the trichloroacetic acid-treated group, tear secretion from the operated eyes also decreased significantly at all time points after operation compared with that preoperatively (Fig. 2). There was also a trend of increasing tear secretion from 1 month (5.81 ± 2.26 mm) to 4 months (8.47 ± 2.76 mm) after surgery.

Tear secretion from the operated eyes was significantly lower in the trichloroacetic acid-treated group than in the non-trichloroacetic acid-treated group at the second month after operation. However, no significant difference in tear secretion was apparent in the operated eyes at the other time points between the two groups.

Ocular Surface Changes

At every month after operation, slit-lamp examination showed abnormal fluorescein and rose bengal staining of the cornea in the operated eyes in both groups (Figs. 3, 4). In comparison with contralateral control eyes, a significant increase of the mean corneal fluorescein-staining and rose bengal-staining scores was found in the operated eyes in both groups at all time points (Figs. 5, 6), but no significant difference occurred in the operated eyes between the two groups ($P > 0.05$). The extent of the corneal staining did not change with time. Although goblet cell densities were lower in the experimental eyes than those in the control eyes in both groups until the fourth month after surgery (Figs. 7, 8), no significant difference was found in the operated eyes between the two groups ($P > 0.05$). Inflammatory pathologic changes of cornea and conjunctiva were not found in either of the eyes in two groups.

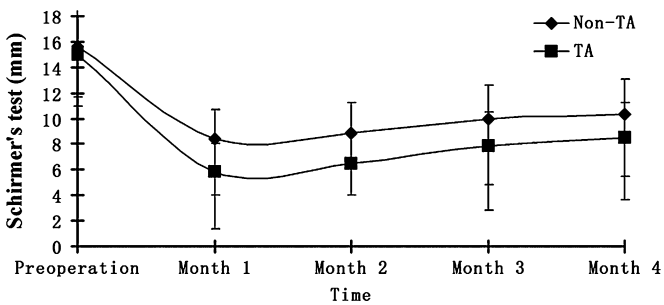

FIGURE 2. Comparison of preoperative and postoperative Schirmer tests in the operated eyes in both groups. Tear secretion from the operated eyes decreased significantly from 1 to 4 months after surgery compared with that before surgery ($P < 0.01$). In each group, at each time point, $n = 10$. Non-TA, non-trichloroacetic acid-treated; TA, trichloroacetic acid-treated.

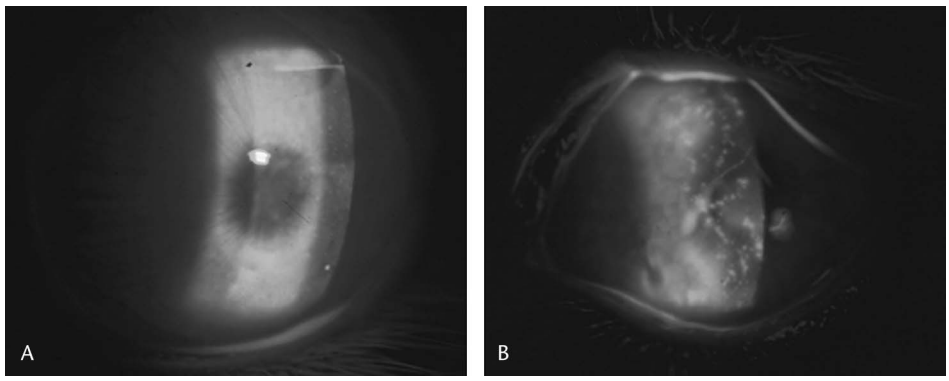

FIGURE 3. Fluorescein staining of the cornea after operation. A, Control eye. B, Operated eye. Diffuse staining can be observed in the operated eye. Control eye shows normal appearance of rabbit cornea.

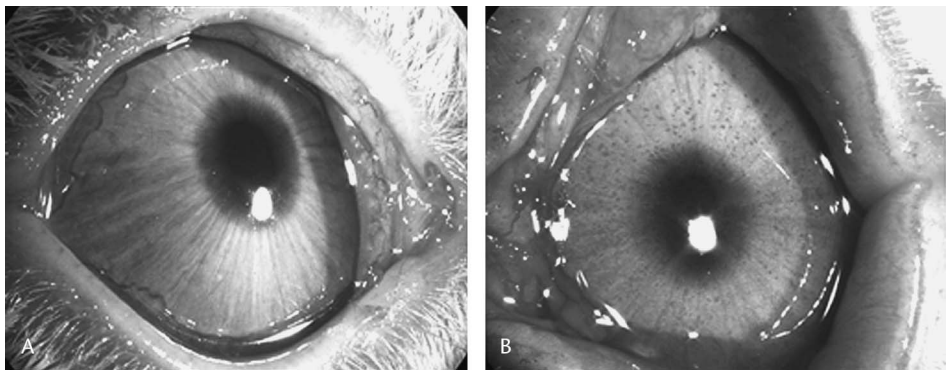

FIGURE 4. Rose bengal staining of the cornea after operation. A, Control eye. B, Operated eye. Diffuse staining can be observed in the operated eye. Control eye shows normal appearance of rabbit cornea.

DISCUSSION

KCS, or dry eye syndrome, results from reduced tear secretion or rapid evaporation of tears.[15] The International Dry Eye Workshop on clinical trials in dry eyes produced a classification that essentially separates dry eye syndrome into two major types on the basis of etiopathogenesis, either aqueous-deficient forms or evaporative forms.[16] In our rabbit model, we reduced tear secretion by removing the lacrimal and harderian glands. Formation of the tear film was prevented by removing the nictitating membrane to reduce blinking.

In past decades, many authors failed to create an ideal rabbit model for KCS surgically because of incomplete removal of the lacrimal and harderian glands. The lacrimal gland is located deep to the orbit and extends along the inferior orbit to the posterior orbit. Although the temporal lobe of the lacrimal gland is superficial, the infraorbital and intraorbital parts of the lacrimal gland are located posteriorly. The harderian gland is located in the anterior corner of eye and extends into the orbital floor. Therefore, these two glands could not be easily and completely ablated because of their anatomy. Many authors removed them through a transconjunctival incision medial to the nictitating membrane to create a rabbit model for KCS. The field of operation could not be well exposed, and the operation was difficult to perform through this approach. Furthermore, bleeding was not easily controlled during the operation because of the abundance of blood vessels around the harderian gland. Hence, it is difficult to ablate the lacrimal and harderian glands completely. Kumar et al[17] described a rabbit model for KCS created by surgically removing the lacrimal gland through a lateral orbital incision. The lacrimal gland could not be removed completely because the infraorbital part of the lacrimal gland was not exposed. The harderian gland also could not be ablated. In this study, we designed a new incision method, which extended from the infraorbital rim to the extraorbital rim. The whole lacrimal gland and harderian gland might be well exposed. This improved visibility of the operative field helped to effectively

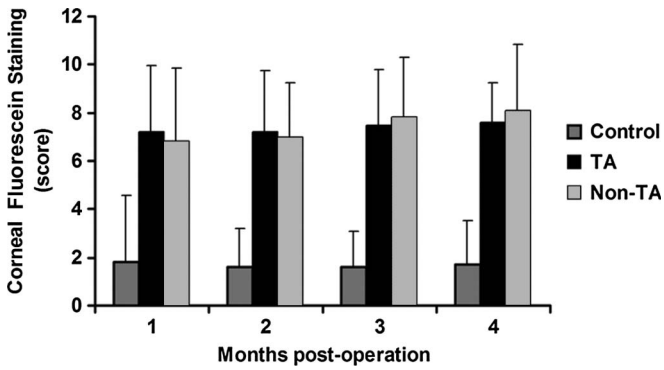

FIGURE 5. Comparison of mean postoperative corneal fluorescein-staining scores between the operated and control eyes. In comparison with contralateral control eyes, a significant increase of mean corneal fluorescein-staining score was seen in the operated eyes in the two groups at every month ($P < 0.01$). In each group, $n = 10$. Non-TA, non-trichloroacetic acid-treated; TA, trichloroacetic acid-treated.

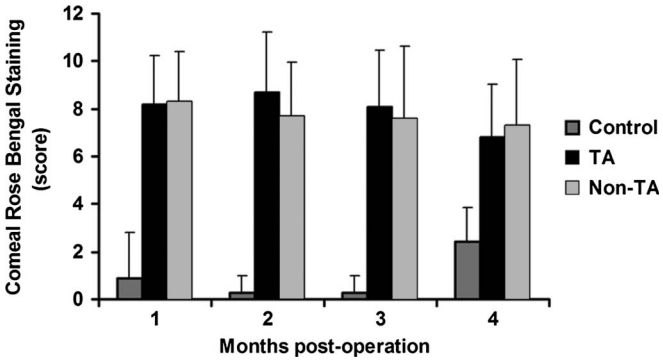

FIGURE 6. Comparison of mean postoperative corneal rose bengal-staining score between operated and control eyes. In comparison with contralateral control eyes, a significant increase of mean corneal rose bengal-staining score was found in the operated eyes in the two groups at every month ($P < 0.01$). In each group, $n = 10$. Non-TA, non-trichloroacetic acid-treated; TA, trichloroacetic acid-treated.

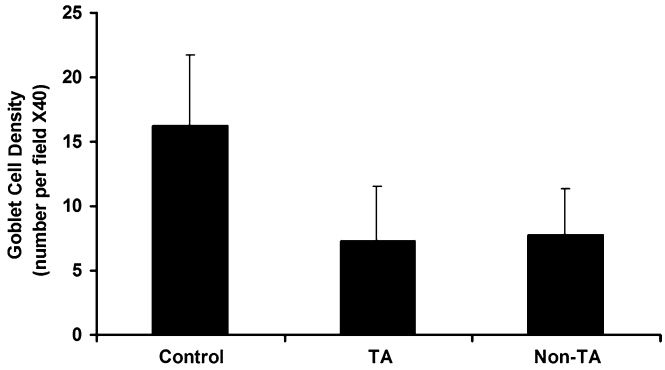

FIGURE 7. Comparison of postoperative mean conjunctival goblet cell density between operated and control eyes. Goblet cell densities were lower in the experimental eyes than those in the contralateral eyes in both groups at the fourth month after surgery ($P < 0.01$). In each group, $n = 10$. Non-TA, non-trichloroacetic acid-treated; TA, trichloroacetic acid-treated.

control the bleeding. Complete ablation of the lacrimal and harderian glands could also be guaranteed.

KCS in humans is frequently accompanied by reduced tear secretion, abnormal corneal staining, and a loss of conjunctival goblet cells. As an indication of tear production, the Schirmer test has an important role in the diagnosis of KCS. Hence, reduced tear secretion might be regarded as a criterion of well-created animal model for KCS. Through a series of experiments since 1987, Gilbard et al[13,18,19] created a rabbit model for KCS by cauterizing the lacrimal gland excretory duct and surgically removing the harderian gland and nictitating membrane. The time span of those experiments ranged from 8 to 52 weeks. Tear film osmolarity increased, whereas corneal epithelial glycogen levels and the conjunctival goblet cell density decreased after surgery. Abnormal corneal rose bengal staining was found at the 44th week postoperatively, although tear secretion did not decrease throughout the experiment. Kumar et al[10] created a rabbit model for KCS by removing the lacrimal gland through a lateral orbital incision. Although corneal ulceration was produced in 55% of the rabbits, reduced tear secretion was not found. In the study of Zhu et al,[20] similar results were published. Sha et al[11] created an ocular surface disease in rabbits by surgically removing the lacrimal gland, the harderian gland, and the nictitating membrane plus transconjunctivally swabbing the palpebral and bulbar conjunctiva with 30% trichloroacetic acid. The conjunctival goblet cell density decreased and corneal rose bengal staining increased at 4 months postoperatively. Moderate and severe inflammatory cell infiltrations of the cornea and conjunctiva were found in 8 of 15 rabbits. However, tear secretion did not decrease after surgery, and it increased at 1 week postoperatively. Jia et al[21] introduced a rabbit model for KCS by surgical removal of the lacrimal gland and swabbing of the palpebral conjunctiva with 20% trichloroacetic acid. Although the tear secretion decreased significantly at 1 month after operation, the long-term result was not evaluated.

In our study, the rabbit model for KCS was created by modifying the previously reported surgical methods. The corneal staining increased significantly at every month, and the conjunctival goblet cell density decreased significantly at the fourth month after surgery in experimented eyes compared with the control eyes. The reduced tear secretion at all postoperative time points sets this KCS rabbit model apart from the previously reported ones, although a gradually recovering trend was noted. Tear secretion might come from

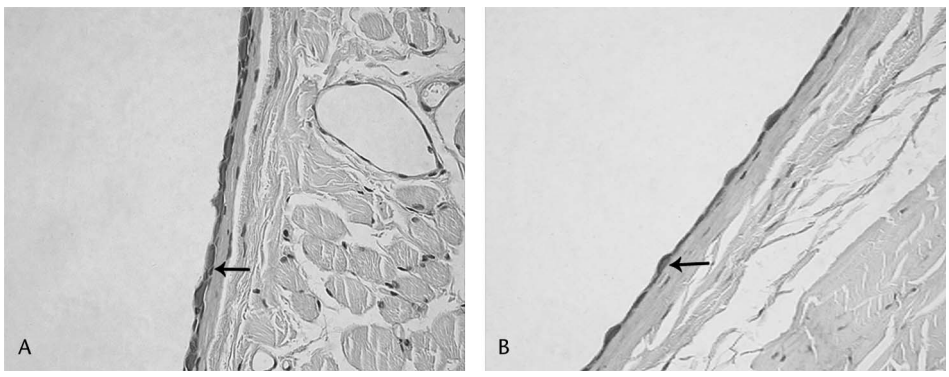

FIGURE 8. Histologic examination of conjunctival goblet cell. A, Control eye. B, Operated eye. The arrowhead is directed at the goblet cell. The goblet cell density is lower in the operated eye than that in the control eye (periodic acid-Schiff; original magnification, ×40).

the accessory lacrimal glands within the tarsal conjunctiva after operation. After comparing two treatment methods applied in this study, we found no significant difference in their effects. The results showed that the burning with a chemical agent did not result in loss of the conjunctival goblet cells. Therefore, it is unnecessary to apply trichloroacetic acid to burn the palpebral and bulbar conjunctiva.

In conclusion, we have successfully established a new rabbit model for KCS by two methods, either of which could be easily and reliably maneuvered. In this model, tear secretion of the operated eyes decreased significantly and remained low over a relatively long time, which is beneficial for studying disease progression and objectively evaluating the therapies for KCS. The achievement of a successful rabbit model for KCS mainly depends on the complete removal of lacrimal and harderian glands.

ACKNOWLEDGMENTS

The authors thank Prof. Bing Li and Mrs. Liao-qing Li, Ophthalmological Institute, Beijing Tongren Hospital, Capital University of Medical Science, for help with the histology and Dr. Eddie C. K. Yau for revision of the manuscript.

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