A New Rabbit Model For Keratoconjunctivitis Sicca

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The authors created a new rabbit model for keratoconjunctivitis sicca by cauterizing the lacrimal gland excretory duct and surgically removing the nictitating membrane and harderian gland. Although the slit-lamp examination findings remained normal for the first 8 wk postoperatively, tear-film osmolarity was elevated by postoperative day 1. Corneal epithelial glycogen levels declined progressively, and conjunctival goblet cell density remained decreased. Multiple controls indicated that closure of the lacrimal gland excretory duct was required for elevation of tear film osmolarity, which, in turn, was required for persistent ocular surface disease. Invest Ophthalmol Vis Sci 28:225-228, 1987

In keratoconjunctivitis sicca (KCS), reduced lacrimal gland secretion results in damage to the ocular surface. This disease is characterized by increased tear-film osmolarity, which may be caused by several mechanisms. In both in vitro and in vivo systems, elevated osmolarity is capable of initiating the ocular surface abnormalities of this disease. Therapies designed to lower tear-film osmolarity have been somewhat successful in controlling the signs and symptoms of KCS.

We report here a new, easily created rabbit model for KCS, which may be helpful in studying the natural history of this disease and objectively evaluating therapies for it.

Materials and Methods

Surgery on Experimental Group

New Zealand white rabbits of both sexes and weighing 2.0–2.5 kg were anesthetized with intramuscular ketamine (100 mg/kg) and xylazine (10 mg/kg). All procedures using animals conformed to the ARVO Resolution on the Use of Animals in Research. With the aid of a microscope, a tapered and polished glass capillary tube was used to locate the lacrimal gland excretory duct. The trocar of a 22-gauge spinal needle was inserted about 2 mm into the duct and then heated using a disposable cautery (Accu-temp, Concept, Inc.; Clearwater, FL) until blanching of the conjunctiva was seen for a distance of about 0.5 mm from the trocar. The trocar was then removed from the duct, and the duct’s orifice, along with a minimal amount of surrounding tissue, was grasped with a jeweler’s forceps. The forceps were then heated. This procedure caused the conjunctiva to fold over the orifice and the fold to stay closed. The fold’s interface was cauterized lightly. A cotton swab was stroked across the cornea, and the area of the excretory duct orifice was observed for lacrimal gland fluid secretion. If secretion was noted, the procedure was repeated. If fluid was not observed, proparacaine was instilled in the eye, and the nictitating membrane containing the nictitans gland was removed by dissecting it sharply at the base. Care was taken not to disturb the orifice of the adjacent lacrimal drainage system. After removing the membrane, the harderian gland appeared spontaneously. Removal of the gland was facilitated by moving the globe temporally with a cotton swab at the limbus.

The procedure was performed in one eye each of nine rabbits, and dexamethasone 0.1% was instilled immediately OU postoperatively and then four times a day for 3 days.

Surgery on Control Groups

Multiple controls were used. In the first control group (eight rabbits), the lacrimal gland excretory duct was cannulated, and then a small inferotemporal area of the conjunctiva remote from the duct was cauterized. This area was equivalent in size to the area cauterized in the experimental group to obtain duct closure.

In the second control group (eight rabbits), proparacaine was instilled in the eye, the nictitating membrane was removed at the base, and the conjunctiva was cauterized as in the first control group.

In the third control group (eight rabbits), the lacrimal gland excretory duct was cannulated and cauterized as in the experimental group. The nictitans and harderian glands were left undisturbed.

All controls were operated in one eye and received dexamethasone 0.1% OU immediately postoperatively and then four times daily for 3 days.
Test Procedures

In the experimental group, slit-lamp examinations and Schirmer tests with proparacaine were performed every other day for the first week and then weekly for 8 wk. In the control groups these examinations were performed weekly for 8 wk.

Tear osmolarity measurements were performed on both eyes of all rabbits as previously described. In the experimental group, measurements were performed every other day for 2 wk and then weekly. In the control groups, measurements were performed on postoperative days 1, 3, and 7, and then weekly. To study conjunctival goblet cell density and corneal epithelial glycogen levels, four rabbits in the experimental group were killed every 2 wk, beginning 4 wk postoperatively, and two rabbits in each control group were killed every 2 wk postoperatively. In the experimental group, measurements of corneal epithelial glycogen levels were omitted at the 2-wk time point.

Since initial studies indicated that both conjunctival goblet cell densities and corneal glycogen levels could vary significantly among rabbits, we expressed these parameters as percentages of values obtained from the contralateral control eyes.

According to Kessing, the conjunctival goblet cell density depends on the topographical location within the conjunctival field. For this reason we obtained conjunctival biopsy specimens at precise, equivalent locations in all eyes. A 5-mm trephine was placed immediately adjacent to the limbus at superonasal, inferonasal, supertemporal, and inferotemporal locations; placement was guided by the location of the extraocular muscles. Conjunctival discs were placed epithelial side up on glass microscope slides coated with gelatin, with the limbal edge oriented up (with the slide frosting on the left). Flat-mount preparations were then fixed and stained as previously described. Goblet cells were counted within a 0.3-mm corridor proceeding from the limbal edge to the edge 180 deg away.

Results

Aside from the absence of the nictitating membrane in some rabbits, no differences were observed among any groups of rabbits in the 8-wk period. Operated eyes were quiet even in the early postoperative period, and control and experimental eyes appeared equally moist. Rabbits commonly showed mild superficial punctate staining of the central cornea with fluorescein dye. All rabbit corneas stained in a characteristic pattern with rose Bengal. Specifically, the cornea was diffusely stained except for a clear zone inferiorly, which ran diagonally across the inferior cornea and enlarged as it continued temporally.

In eyes with the lacrimal gland excretory duct occluded and the nictitating membrane and harderian glands removed, Schirmer tests with proparacaine averaged 6.87 ± 0.4 mm (SEM) vs. 7.54 ± 0.5 mm (SEM) in contralateral control eyes (n = 72), but the difference was not significant. Nevertheless, tear-film osmolarity was elevated as early as postoperative day 1 and remained elevated relative to the contralateral control eyes. During the 8-wk period, tear osmolarity in the experimental eyes averaged 321 ± 0.9 mOsm/L (SEM) compared with 303 ± 0.3 mOsm/L (SEM) in contralateral control eyes (P < 0.01) (Fig. 1). Goblet cell den-
Fig. 2. Goblet cell density (four bulbar quadrants pooled) in rabbit models relative to contralateral control eyes over 8 wk. Abbreviations: NM, nictitating membrane; HG, harderian gland; LGD, lacrimal gland excretory duct; conj, conjunctiva.

Figures shows the goblet cell density (four bulbar quadrants pooled) in rabbit models relative to contralateral control eyes over 8 wk. Abbreviations: NM, nictitating membrane; HG, harderian gland; LGD, lacrimal gland excretory duct; conj, conjunctiva.

Goblet cell densities were lower in experimental eyes than in contralateral control eyes throughout the 8-wk period (Fig. 2). In the experimental group, postsurgical corneal epithelial glycogen levels decreased progressively relative to contralateral control eyes (Table 1).

In the first control group, in which a conjunctival burn was created in an inferotemporal location remote from the lacrimal gland excretory duct, tear osmolarities averaged $304 \pm 0.2$ mOsm/L (SEM) (Fig. 1), and goblet cell densities increased and decreased minimally during the 8-wk period and stabilized to 98% of the control group by 8 wk (Fig. 2). Glycogen levels did not decrease in these rabbits at any time point (Table 1).

In the second control group with the nictitating membrane removed and the conjunctiva cauterized inferotemporally, tear osmolarities averaged $305 \pm 0.35$ mOsm/L (SEM) (Fig. 1), and goblet cell densities decreased initially but returned to normal by 8 wk (Fig. 2). Corneal glycogen levels showed progressive recovery throughout the 8-wk period (Table 1).

In the third control group, in which the only manipulation was closure of the lacrimal gland excretory duct, tear osmolarities increased by postoperative day 1. For the 8-wk period, they were significantly increased relative to controls, averaging $313 \pm 0.5$ mOsm/L (SEM) ($P < 0.01$) (Fig. 1). Goblet cell densities and corneal epithelial glycogen levels were not studied in this group. Rabbits were saved for study at later time points.

**Discussion**

We report here a surgically created rabbit model for KCS characterized by increased tear-film osmolarity, decreased conjunctival goblet cell density, and decreased corneal epithelial glycogen levels. It is well documented that tear-film osmolarity is increased\textsuperscript{1,2,8} and goblet cell density is decreased in KCS.\textsuperscript{13-15} Corneal epithelial glycogen levels have not been measured in this disease. In our rabbit model, the slit-lamp examination and Schirmer test results remained normal for 8 wk.

Given that the orbital lacrimal gland excretory duct was closed, and the nictitans and hardarian glands were removed in our experimental model, the fluid in the tear film may have come from the accessory lacrimal glands within the tarsal conjunctiva\textsuperscript{16} as well as from fluid transported across the conjunctiva and cornea.\textsuperscript{17,19} It is evident that our procedure for closing the lacrimal excretory duct in the rabbits was effective, given the results from our third control group in which this procedure alone led to an increase in tear-film osmolarity throughout the 8-wk period.

Removing the nictitating membrane with its nictitans gland and the hardarian gland, along with closing the lacrimal gland excretory duct, led to a greater increase in tear-film osmolarity than closing the lacrimal gland excretory duct alone. Nevertheless, removing the nictitating membrane was not sufficient to elevate osmolarity.

**Table 1.** Corneal epithelial glycogen levels after surgery—mean % decrease relative to contralateral control eyes

<table>
<thead>
<tr>
<th>Time after surgery (wk)</th>
<th>Experimental rabbit group</th>
<th>First control rabbit group</th>
<th>Second control rabbit group</th>
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<tr>
<td>2</td>
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molarity from the normal range or induce ocular surface disease as indicated by the goblet cell density or corneal epithelial glycogen level. Ocular surface disease in the experimental model was not caused by an "exposure" problem related to the absence of the nictitating membrane but developed only in the experimental rabbits with elevated tear-film osmolarities. We attribute early goblet cell and glycogen abnormalities in the controls to surgical stress, the ocular surface, as indicated by these criteria, recovered fully by 8 wk postoperatively. Because tissue changes due to surgical trauma can be a confusing variable, the experimental model for KCS should not be used as a model for the ocular surface disease of KCS until 8 wk postoperatively.

In the past, Beitch described a rat model for KCS created by surgically removing the exorbital lacrimal gland. Two other potential animal models have also been reported: Kessler and co-workers described ocular findings in the NZB/NZW F1 hybrid mouse, and Maudgal created ocular surface disease in the rabbit by surgically removing the orbital lacrimal gland as well as the hardierian and nictitans glands. In an earlier study we observed lacrimal gland infiltration in the NZB/NZW F1 hybrid mouse but no abnormalities of the tear film or ocular surface. The surgically altered rabbits in Maudgal's study had features highly unusual for KCS, including epithelial and stromal hazeiness; more than lacrimal secretion may have been altered. Furthermore, we found Maudgal's operation extraordinarily difficult to perform and traumatic to the lids.

Our new rabbit model for KCS is easily and reliably created, and may be useful in studying the natural history of this disease and objectively evaluating therapies.

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

References