Corneal Endothelial Function and Structure Following Cryo-Injury in the Rabbit


Wide-field specular microscopy, fluorophotometry, pachymetry, and scanning electron microscopy are used to characterize a reproducible, in vivo model of corneal endothelial injury and recovery in the rabbit. Following an 8-mm central cryo-injury, the cornea remains thickened for as long as 3 weeks. Mean endothelial permeability to fluorescein is above normal for 10 days following injury, but by 14 days postinjury the endothelial permeability to fluorescein is not statistically significantly different from preinjury control values, thus indicating that endothelial permeability probably returns to normal by approximately 2 weeks postinjury. Cell morphology, as determined by scanning electron microscopy, is also essentially normal by 2 weeks postinjury. Endothelial permeability appears to recover before stromal thickness normalizes, suggesting a lag in recovery of endothelial pump function. Invest Ophthalmol Vis Sci 25:1416–1425, 1984

Dysfunction of the corneal endothelium resulting in clinically significant stromal and epithelial edema can be caused by endothelial dystrophy, inflammation, drugs, glaucoma, trauma, and surgery. Recently, surgical advances such as phacoemulsification, extracapsular cataract surgery, and intraocular lenses have been shown to cause important endothelial cell loss.1–3 Because current medical management of endothelial cell loss and physiologic function is limited, the development of a model for pharmacologic manipulation of endothelial function is of increasing clinical relevance.

Several investigators have studied corneal thickness and endothelial morphology following cryo-injury. In rabbit cornea, Khodadoust and Green4 demonstrated that following a single 4-mm diameter central cryo-injury, central corneal thickness increased and then returned to normal after 10–12 days. However, the denuded area of Descemet’s membrane was recovered by endothelium after 3 days, suggesting an abnormality of the intercellular junctions as the etiology of the increased corneal thickness. Van Horn et al.5 using a double freeze-thaw, destroyed 10, 50, or 90% of the endothelium and studied endothelial cell division, corneal thickness, and endothelial morphology in rabbits and cats. They found that in rabbits, extensive cellular division, as measured by autoradiographic analysis of the incorporation of 3H-thymidine into DNA, occurred after a 50% injury. Furthermore, the endothelial cells reformed a monolayer of normal density and morphology by 10 days, but corneal thickness did not return to normal until 28 days. Staatz and Van Horn subsequently demonstrated that endothelial wounds healed in older rabbits more slowly than in younger rabbits.6

To study the effects of various pharmacologic agents on endothelial physiology during wound healing in vivo, we have developed a reproducible model of endothelial dysfunction induced by transcorneal cryo-injury. The wounded corneas are studied using wide field specular microscopy, fluorophotometry, pachymetry, and scanning electron microscopy.

Fluorophotometry provides a method for determining the permeability to fluorescein across the corneal endothelium in vivo,7,8 and these measurements correlate with endothelial barrier function. For example, Burns, Bourne, and Brubaker9 used fluorophotometry to demonstrate increased permeability to fluorescein and inferred that endothelial pump function was normal in a series of patients with Fuchs’ dystrophy. We have chosen to apply these techniques to the rabbit cornea. Although Van Horn et al suggested the cat as a model for endothelial loss comparable to the human,3 cats often have extensive and persistent corneal edema which would preclude

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wide field specular microscopy and fluorophotometry. In the mature rabbit, however, following transcorneal freezing, endothelial recovering occurs and, importantly, the cornea becomes clear enough for specular microscopy and fluorophotometry.

Materials and Methods

General

Before and after unilateral wounding, bilateral measurements of central corneal thickness, endothelial density, and cornea-to-aqueous mass transfer coefficient were made in sedated (60 mg/kg of Ketamine and 40 mg/kg of Thorazine IM), adult, pigmented rabbits weighing 3.5–5.0 kg. Postinjury measurements were grouped into the following periods: 8–10 days, 12–14 days, and after 21 days. Five of 17 rabbits died before or during postinjury measurements are are included only in the initial data. All animals were utilized in accordance with the ARVO Resolution on the Use of Animals in Research.

Wounding

The central cornea of one eye was treated once for 15 sec by immediately placing on the eye an 8-mm diameter brass dowel that had been cooled in liquid nitrogen. The end of the dowel was concave (radius: 8 mm). Saline irrigation was used to thaw the aqueous iceball.

Pachymetry

Central corneal thickness, \( q \), was measured with a Maurice-Giardini pachymeter calibrated against hard contact lenses of known thicknesses. A linear correction factor of 1.115 was found for our particular instrument. Measurements were made before iontophoresis.

Specular Microscopy

The Keeler/Konan wide-field specular microscope (total field size: 0.8 by 1.2 mm) was used to photograph the endothelium. When the cornea was relatively clear, only central photographs were taken. When the central corneal specular image was of poor quality, due to the swollen stroma and an irregular endothelial pattern 8–10 days postinjury, an average endothelial density was determined using four separate areas from nasal and temporal regions directly adjacent to the central injury area.

Fluorophotometry

Following one drop of 0.5% proparacaine, 2% fluorescein was iontophoresed for 5–7 sec into the central cornea from either a 4 mm agar or hydrogel probe. Within 20 min, the mass of fluorescein in the cornea and the cornea/aqueous spread function were measured. Central corneal and aqueous fluorescein concentrations were determined periodically between 90 and 500 min after iontophoresis, usually every 60–90 min. Throughout the course of the experiment, the ocular surface was lubricated sparingly with either balanced salt solution or 1% methylcellulose.

The fluorophotometer used for these experiments has a slit height of 3.0 mm, illuminating beam width (\( w_i \)) of 250 \( \mu \)m, and detection beam width (\( w_d \)) of 130 \( \mu \)m (angle: 50°). The fluorophotometer is similar to that described elsewhere and uses a regulated incandescent light source. Anterior chamber volumes were determined by paracentesis of the uninjured eye immediately after each rabbit was killed. No effort was made to aspirate posterior chamber fluid. The volume, determined gravimetrically, was used in calculations for both eyes.

Method 5 of Coakes and Brubaker was used to analyze the data. At each measurement after iontophoresis, the mass of fluorescein present in the anterior chamber was calculated from the aqueous fluorescein concentration (corrected for the cornea/aqueous spread function) and the anterior chamber volume. The time course of the change in fractional fluorescein mass was fit (equal weighting) to the two compartment model of fluorescein kinetics with computer aided (direct search) least-squares analysis, and the cornea-to-aqueous mass transfer coefficient, \( k_{ca} \), was calculated. When calculating \( k_{ca} \), the apparent corneal volume of fluorescein distribution of the rabbit cornea was assumed to be 80 \( \mu \)l and independent of stromal swelling (see Appendix for justification). Endothelial permeability to fluorescein, \( K \), was calculated from the formula:

\[
K = \frac{k_{ca} \cdot q}{r_{ac}}
\]  

where \( q \) is corneal thickness, and \( r_{ac} \) is the aqueous-to-cornea distribution ratio. We used a value of 0.45 for \( r_{ac} \).

Morphology

At various intervals, animals were killed with an overdose of intravenous pentobarbital, and corneas were immediately placed in half-strength Karnovsky's fixative for at least 24 hr. Whole corneas were dehydrated in a graduated series of ethyl alcohol, critical point dried, sputter coated with gold palladium (Polaron E5100), and examined using a scanning electron microscope (AMR 1000A).
Table 1. Pre- and postinjury means ± SD for endothelial density, fluorescein mass introduced by iontophoresis, corneal thickness (q), cornea-to-aqueous mass transfer coefficient (kca), and endothelial permeability to fluorescein (K)

<table>
<thead>
<tr>
<th>Time</th>
<th>No. of eyes</th>
<th>(Cells/mm²)</th>
<th>Mass (ng)</th>
<th>q (mm)</th>
<th>kca (10⁻³/min)</th>
<th>K (10⁻⁴ cm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinjury</td>
<td>31</td>
<td>2763 ± 306</td>
<td>170 ± 152</td>
<td>0.44 ± 0.05</td>
<td>2.7 ± 1.2</td>
<td>2.6 ± 1.2</td>
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<tr>
<td>1–7 days</td>
<td>9</td>
<td>no data</td>
<td>no data</td>
<td>&gt;0.9</td>
<td>no data</td>
<td>no data</td>
</tr>
<tr>
<td>8–10 days</td>
<td>6</td>
<td>1529 ± 410*</td>
<td>281 ± 192</td>
<td>0.57 ± 0.06*</td>
<td>4.5 ± 1.8*</td>
<td>5.6 ± 1.7*</td>
</tr>
<tr>
<td>12–14 days</td>
<td>7</td>
<td>2156 ± 487*</td>
<td>142 ± 146</td>
<td>0.52 ± 0.10*</td>
<td>2.8 ± 1.0</td>
<td>3.2 ± 1.0</td>
</tr>
<tr>
<td>21–40 days</td>
<td>5</td>
<td>2868 ± 554</td>
<td>270 ± 155</td>
<td>0.44 ± 0.02</td>
<td>3.1 ± 0.9</td>
<td>3.0 ± 0.8</td>
</tr>
<tr>
<td>Group C†</td>
<td>16</td>
<td>2910 ± 403</td>
<td>178 ± 138</td>
<td>0.45 ± 0.04</td>
<td>3.2 ± 1.7</td>
<td>3.3 ± 1.8</td>
</tr>
</tbody>
</table>

* P < 0.01 (Student's t-test).
† Group C is formed by the uninjured contralateral eyes of the 14 experiments shown and 2 additional experiments.

Results

Corneal Thickness

As shown in Table 1, mean corneal thickness was 0.44 mm before wounding. Twenty-four hours after injury, mean corneal thickness had increased to more than two times the normal value. Between 4 and 21 days, as epithelial and endothelial wounds closed, mean corneal thickness decreased. By 8–10 days postinjury, mean corneal thickness had decreased to 0.57 mm, and little further change occurred over the next several days. By three weeks, mean corneal thickness had returned to preinjury values.

Endothelial Density

Mean endothelial cell density prior to wounding was 2,763 cells/mm² (Table 1). Immediately after freezing, a well demarcated area of endothelial damage was observed by specular microscopy (Fig. 1A). For the next 7 days, specular microscopy was not possible due to corneal clouding. By 8–10 days, use of the specular microscope was again possible, and the mean endothelial cell density was found to be reduced to 1,529 cells/mm². Between 12 and 14 days postinjury, mean endothelial cell density was 2,156 cells/mm² (Fig. 1B). Subsequently, by 3–4 weeks postinjury, mean endothelial cell density returned to control values (Fig. 1C).

Transfer Coefficient and Permeability

The mean masses of fluorescein introduced by iontophoresis are presented for each period in Table 1. Although the means fall between 142 and 281 ng, considerable variability existed with an overall range between 40 and 650 ng. Experiments in which the mass was less than 40 ng or greater than 650 ng were excluded because of problems calibrating mass measurements for very low or very high mass values. Anterior chamber volumes were between 300 and 400 µl (data not shown). The cornea-to-aqueous mass transfer coefficients, kca, are grouped into preinjury values, postinjury values, and values from the uninjured contralateral eyes measured throughout the postinjury period (group C of Table 1). The mean cornea-to-aqueous mass transfer coefficient, kca, before injury was 2.7 x 10⁻³ min⁻¹. The transfer coefficient was not accurately measurable during the first 7 days postinjury, but presumably kca increases dramatically in the absence of endothelium. By 8–10 days postinjury, the mean transfer coefficient was significantly increased to 4.5 x 10⁻³ min⁻¹ (P < 0.01, Student's t-test), but had returned to control values by 12–14 days postinjury.

Endothelial permeability to fluorescein was calculated from kca, q, and rca for each experiment according to Equation 1, and the means for each period are shown in Table 1. Initially, mean endothelial permeability was 2.6 x 10⁻⁴ cm/min. Between 8 and 10 days postinjury, permeability was significantly increased to 5.6 x 10⁻⁴ cm/min (P < 0.001, Student's t-test). Between 12 and 14 days postinjury, mean endothelial permeability was 3.2 x 10⁻⁴ cm/min. This value is statistically significantly lower than the 8–10 day permeability value (P < 0.01, Student's t-test). The 95% confidence interval is 0.7 to 4.1 x 10⁻⁴ cm/min. Furthermore, although higher than the control value, the 12–14 day permeability value is not significantly different (P > 0.1, Student's t-test). The 95% confidence interval for the difference between the mean of the preinjury controls and the mean of the 12 to 14 day permeabilities is −0.4 to 1.6 x 10⁻⁴ cm/min. Late endothelial permeability was also not significantly different from the initial value.

Mean endothelial permeability for all uninjured contralateral eyes (group C) was 3.3 x 10⁻⁴ cm/min. This value is not statistically different from the preinjury level (P > 0.05, Student's t-test). Group C is composed of three groups of uninjured contralateral
eyes (measured between 0 and 10 days, 12 and 14 days, and later than 21 days after wounding of fellow eyes) having mean permeabilities, respectively, of 3.2 (seven eyes), 3.0 (three eyes), and 3.5 (six eyes) \( \times 10^{-4} \) cm/min.

**Morphology**

Before wounding, the endothelial cells exhibited the typical hexagonal shape, and along each intercellular edge five to seven overlapping interdigitations of the lateral cell membranes were each approximately twice as long as wide (Fig. 2). After injury, surviving cells migrated towards the center of the wound and appeared elongated with abundantly free surface membrane ruffling and long cytoplasmic projections extending towards, and often over, other cells (Fig. 3). Marked pleomorphism was noted. Three to 4 days after injury, the entire wound area was covered completely by an irregular endothelial layer.

Six days after injury, cells were more flattened, large, and irregular in shape. Less surface membranous and filipodial projections were seen (Fig. 4). By 8–10 days after injury, although still relatively large, endothelial cells began to appear more hexagonal. Instead of long cytoplasmic projections, numerous fingerlike interdigitations were observed in some areas (Fig. 5). By 12–14 days, hexagonal morphology was restored, and cell surfaces appeared normal; however, the cells appeared somewhat pleomorphic and enlarged to approximately 20–25 \( \mu \)m in diameter (Fig. 6A). Cell borders showed attenuated interdigitations in some areas (Fig. 6B). Finally, after 4 weeks, cell density and morphology appeared normal (Fig. 6C).

**Discussion**

We have described an in vivo model of corneal endothelial wound healing in the adult, pigmented rabbit. Following cryo-injury, stromal thickness in-
increases dramatically and then gradually returns to normal over a 3-week period. Initially, swelling is due to both epithelial and endothelial abnormalities. However, the epithelium heals within the first 5 days (data not shown), and, in any event, active epithelial transport contributes only minimally to stromal dehydration. The stromal swelling that persists beyond the first week is undoubtedly due to endothelial dysfunction.

The time course of the rabbit model of endothelial injury and recovery can be divided into five periods based on differences in corneal thickness, permeability to fluorescein, and morphology (Fig. 7).

Before Injury

Prior to injury, our values for mean corneal thickness of 0.44 mm and for endothelial density of 2,763...
Fig. 4. Six days after injury. Scanning electron micrograph shows marked pleomorphism. In some instances, cell surfaces exceed 50 μm in diameter (arrowheads, bar = 10 μm). Compared with 3 days after injury (Fig. 3), fewer surface membrane and filipodial projections are noted.

Fig. 5. Nine days after injury. Left. Scanning electron micrograph demonstrates enlarged cells with approximate diameters of 50 μm or less, but numerous cells begin to appear polygonal (bar = 10 μm). Right. At higher magnification, the intercellular junctions are characterized by long fingerlike interdigitations in some areas (bar = 10 μm).
cells/mm² are similar to those reported in the literature. Our value of $2.6 \times 10^{-4}$ cm/min for endothelial permeability to fluorescein in normal, pigmented rabbit cornea is somewhat less than, but generally comparable with, that obtained by Mishima and Maurice¹³ using iontophoresis ($3.6 \times 10^{-4}$ cm/min) and Ota et al.¹⁴ using intravenous administration ($3.0 \times 10^{-4}$ cm/min).

**Zero to Four Days Postinjury**

By 24 hr after wounding, corneal thickness increased to more than twice normal; the marked stromal swelling precluded specular microscopy and accurate fluorophotometry. In selected animals, however, scanning electron microscopy demonstrated that the wounds were covered coarsely by 3–4 days postinjury, but there was a lack of adequate intercellular contact as indicated by long cytoplasmic projections (Fig. 3). Van Horn et al also found that an 8 mm diameter central endothelial wound was recovered coarsely by 5 days postinjury.² Because of the incomplete barrier, endothelial permeability to fluorescein presumably increases and endothelial pump function decreases in response to the endothelial injury.

**Eight to Ten Days Postinjury**

Despite reformation of the endothelial monolayer 3–4 days postinjury, 8 days postinjury was the earliest that we could perform specular microscopy and fluorophotometry. At this time, mean corneal thickness was increased by 30%, and endothelial density was decreased by 45%. Because we were not always able to photograph adequately the abnormal endothelium during this period, paracentral photographs probably overestimated mean central endothelial density given in Table 1. In general, our results for corneal thickness and endothelial density are consistent with those reported previously.⁵,⁶

Endothelial permeability increased by 115%. Morphologic analysis of the endothelial monolayer in this
period is consistent with increased permeability; the long fingerlike cellular interdigitations suggest the lack of normal intercellular contacts (Fig. 5).

Twelve to Fourteen Days Postinjury

When measured between 12 and 14 days postinjury, corneal thickness was increased by 18% of normal, and cell density was decreased by 22% of normal. Endothelial permeability to fluorescein, however, was not significantly different from the control value during this period.

It appears that endothelial permeability to fluorescein recovers before stromal thickness. We assume that fluorescein is not actively transported, and that permeability to fluorescein is proportional to permeability to water. The difference in recovery between permeability and thickness therefore suggests that endothelial pump function recovers to produce a dehydrated stroma after endothelial permeability has returned to normal. However, measurements of permeability are quite variable and so a small increase in endothelial permeability to fluorescein might be missed. Given our sample sizes, the 12–14 day permeability value could have been $3.6 \times 10^{-4}$ cm/min before a statistical difference ($P < 0.05$) would have been detected. Furthermore, fluorescein is a larger molecule than either water or the common electrolytes and might fail to permeate a slightly disrupted paracellular pathway. Thus, the possibility remains that the increased stromal thickness resulted from a slightly elevated endothelial permeability which was not detected with fluorophotometry because of either method variability or the relatively larger size of fluorescein.

During this period, the return of the hexagonal cell appearance was documented with wide field specular microscopy, and the interendothelial cell junctions appeared normal by scanning electron microscopy. Transmission electron microscopy will be used to determine the actual morphology of the intercellular junctions. Normal endothelial permeability, as measured by fluorophotometry, is thus consistent with the morphologic appearance of the tissue at this time. Our data suggests, but does not prove, that the appearance of hexagonal morphology implies normal permeability (Fig. 6).
Twenty-one or More Days Postinjury

By 3 weeks after injury, mean endothelial permeability to fluorescein and corneal thickness have both returned to preinjury values. This time course of healing is generally consistent with the return to normal values for corneal thickness reported by Van Horn et al.\(^5\) The central endothelial cell density 28 days after injury has returned to preinjury values and was actually greater than the individual preinjury value in a few animals. The return to preinjury values is consistent with endothelial mitosis. The morphology of selected animals was remarkable only for its apparent normality (Fig. 6C).

We recognize that there are several possible errors in the methods used for deriving transfer coefficients and permeability:

1. The broad range of fluorescein masses that were observed (40–650 ng) contributed to overall variability in the transfer coefficients. Therefore, paired comparisons of before and postinjury data are not meaningful, and only the means of the various periods should be compared.

2. The resolution of the fluorophotometer was relatively large (in air), and this may have underestimated, especially in thinner corneas, the measured concentration of fluorescein in the cornea relative to the anterior chamber. Because the cornea/aqueous spread function was derived with the same resolution, we assumed that the two errors offset each other, regardless of whether the cornea was of increased or normal thickness.

3. We used an aqueous-to-cornea fluorescein distribution ratio (\(r_{ac}\)) of 0.45 for both normal and swollen corneas. Most authors use \(r_{ac} = 0.6\), regardless of corneal swelling.\(^8,9\) After injecting 1.0 mg of sodium fluorescein in 0.05 ml saline into the vitreous of both eyes in pigmented rabbits, simultaneous corneal and aqueous fluorescein concentrations were measured in vivo after the injection, and \(r_{ac}\) was derived. Mean \(r_{ac}\) was 0.36 ± 0.04 (n = 20 eyes) at 48 hr; 0.45 ± 0.08 (n = 8 eyes) at 54 hr; and 0.45 ± 0.03 (n = 12 eyes) at 72 hr.\(^12\) These data indicate that in normal cornea, \(r_{ac}\) is closer to 0.45 than to 0.6.

4. Aqueous protein induced by transcorneal freezing may bind fluorescein and alter kinetics. However, Buco et al have already shown that aqueous protein returns to control levels by 7 days postinjury\(^14\) (our own studies confirmed their findings—data not shown). Therefore, we used no correction factor for fluorescein binding to aqueous protein.

5. Because differences in anterior chamber volume make only a minimal difference in the calculation of \(k_{ac}\),\(^11\) we used aspiration to estimate true anterior chamber volume. We assumed that no significant change in volume occurred secondary to freezing, that anterior chamber volumes remained constant throughout all experiments, and that the volumes of the right and left eyes were similar.

6. We assumed that the fluorescein concentration detected in the anterior chamber was not significantly attenuated by scatter in the swollen cornea. Such an assumption is supported by comparisons of calibrated fluorophotometer measurements of in vivo aqueous fluorescein concentration to in vitro fluorescein determinations in the same fluid as obtained by aspiration (unpublished data). If, however, scatter were significant, then measured aqueous fluorescence would be artificially decreased, but corneal fluorescence would be closely approximated. Consequently, cornea/aqueous spread function, although higher in swollen corneas, would be accurate since no fluorescein is in the aqueous when cornea/aqueous spread function is measured. Thus, the calculated aqueous fluorescein concentration obtained by subtracting an accurate cornea/aqueous spread function from an artificially decreased measured aqueous fluorescein concentration, would be artificially decreased, especially during early time points. This would reduce \(k_{ac}\) and hence underestimate derived permeability. In other words, attenuation of the aqueous fluorescein signal by corneal scatter could induce an artificially low but not a high permeability measurement. Our results are in the opposite direction.

The return-to-normal curves for endothelial permeability, corneal thickness, and endothelial density are shown schematically in Figure 7. Healing can be divided into four phases: (1) During the initial phase, peripheral endothelial cells migrate and undergo mitosis to recover the denuded area with an (incomplete) endothelial monolayer. (2) The second phase is characterized by increased endothelial permeability, while the cells restore their intercellular junctions to more normal structure. (3) During the third phase, endothelial permeability appears normal, but the cornea remains swollen, suggesting a lag in recovery of endothelial pump function. (4) Finally, in the fourth phase, normal thickness is restored. This work does not include transmission electron microscopy, and so the exact structural basis for the observed physiologic changes awaits further study.

Key words: corneal endothelium, endothelial permeability, fluorophotometry, scanning electron microscopy, specular microscopy

Appendix

For calculations of \(k_{ac}\), we chose 80 µl as the apparent corneal volume of fluorescein distribution in the rabbit.
The apparent corneal volume of fluorescein distribution at corneal thickness, \( q \), \( V_{c}^{\text{dist}}(q) \), may be expressed as follows:

\[
V_{c}^{\text{dist}}(q) = \frac{v_c(q)}{r_{ec}}
\]

(2)

where \( v_c(q) \) is the volume of the cornea at corneal thickness \( q \), and \( r_{ec} \) is the aqueous-to-cornea fluorescein distribution ratio at equilibrium.

\[
v_c(q) \sim \pi \cdot (d/2)^2 \cdot q
\]

(3)

where \( d \) is the diameter of the cornea.

For two compartment modeling of fluorescein kinetics, an average effective corneal diameter is used. The effective corneal diameter is determined by the area of endothelium essentially the depot size. Late in the experiment, after the depot has diffused laterally, and the peripheral cornea has essentially the depot size, and negligible fluorescein is in the anterior chamber, the effective corneal diameter is the deposit size. Late in the experiment, after the depot has diffused laterally, and the peripheral cornea has been exposed to aqueous fluorescein as well, the effective diameter approaches the total diameter of the cornea. We assumed an average effective corneal diameter of 1.0 cm for the normal rabbit. Thus in normal rabbit cornea:

\[
V_{c}^{\text{dist}}(q) \sim \frac{\pi \cdot 0.25 \text{ cm}^2 \cdot q}{r_{ec}} \\
\sim 80 \mu l
\]

from equations 2, 3, and \( r_{ec} = 0.45 \),\(^{11} \)

The question arises as to whether variations in \( V_{c}^{\text{dist}} \) have a significant effect on the calculated \( k_a \) generated by a least squares fit of the data, not only as pertains to the preceding analysis of normal conditions but especially to conditions where the cornea is experimentally swollen (increased \( V_{c}^{\text{dist}} \)). (Note that for our purposes the question of whether \( r_{ec} \) depends on \( q \) can be included in the preceding more general question regarding \( V_{c}^{\text{dist}} \).) To study this question, we generated idealized fractional mass data points for the 8 hourly time points between 0 and 420 min by setting \( V_{c}^{\text{dist}} \) equal to 80 \( \mu l \) and for three \( k_a \) conditions: low, normal, and high values (1.00, 2.50, and 5.00 \( \times 10^{-3} \text{ min}^{-1} \)).\(^* \) Then \( V_{c}^{\text{dist}} \) was varied between 50 and 250 \( \mu l \) and the idealized fractional mass data was refit thereby generating new \( k_a \) and \( k_e \) values. Meanwhile, anterior chamber volume was held fixed at 250 \( \mu l \) for all cases. The variations in \( k_a \) and \( k_e \) as a function of \( V_{c}^{\text{dist}} \) for the three \( k_a \) conditions are shown in Table 2, and it can be seen that a variation in \( V_{c}^{\text{dist}} \) from 50–125 \( \mu l \) produces less than a 3% change in \( k_a \) under the three conditions specified. Furthermore, it is evident that an underestimation of \( V_{c}^{\text{dist}} \) artificially generates a slightly lower \( k_a \) value, especially when \( k_a \) is already increased. In our study, such an effect, if significant, only pertains when both thickness and \( k_a \) are significantly increased. By using 80 \( \mu l \), we probably underestimated \( V_{c}^{\text{dist}} \) and hence underestimated the already significantly increased \( k_a \) when measured between 8–10 days postinjury.

Table 2. Variation in \( k_a \) and \( k_e \) as a function of \( V_{c}^{\text{dist}} \) for fractional mass data generated under three conditions of \( k_a \)

<table>
<thead>
<tr>
<th>( V_{c}^{\text{dist}} )</th>
<th>( k_a )</th>
<th>( k_e )</th>
<th>( k_{ch} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 250 )</td>
<td>313.1</td>
<td>1.02</td>
<td>2.0 +0.2</td>
</tr>
<tr>
<td>( 125 )</td>
<td>156.0</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>( 80^* )</td>
<td>100.0</td>
<td>1.00</td>
<td>0</td>
</tr>
<tr>
<td>( 50 )</td>
<td>63.0</td>
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</tr>
<tr>
<td>( 250 )</td>
<td>313.2</td>
<td>2.60</td>
<td>+4.0</td>
</tr>
<tr>
<td>( 125 )</td>
<td>156.2</td>
<td>2.52</td>
<td>+0.8</td>
</tr>
<tr>
<td>( 80^* )</td>
<td>100.2</td>
<td>2.50</td>
<td>0</td>
</tr>
<tr>
<td>( 50 )</td>
<td>63.2</td>
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<td>( 250 )</td>
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<tr>
<td>( 125 )</td>
<td>156.5</td>
<td>5.14</td>
<td>+2.8</td>
</tr>
<tr>
<td>( 80^* )</td>
<td>100.5</td>
<td>5.00</td>
<td>0</td>
</tr>
<tr>
<td>( 50 )</td>
<td>63.5</td>
<td>4.90</td>
<td>-2.0</td>
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</table>

\(^* \) For each of the three \( k_a \) conditions, the anterior chamber elimination coefficient, \( k_e \), was set to 1.50 \( \times 10^{-2} \text{ min}^{-1} \).