Corneal Edema Recovery Dynamics in the Rabbit

A Useful Model?

Peter R. Herse

Open-eye and closed-eye recovery from contact lens-induced corneal edema was measured on the right eyes of 10 New Zealand White rabbits using ultrasound pachometry. Edema was produced by either 2 hours of eye closure (Patch Test) or 2 hours wearing of a thick hydrogel contact lens over the closed eye (Lens Test). The measured corneal edema recovery data were analyzed by both linear-regression analysis and nonlinear-regression analysis based on a previously published exponential open-eye edema recovery model. Linear-regression analysis revealed that the initial rabbit open-eye edema recovery rate of 36.3 ± 11.9 (standard deviation) μm/hr was not significantly different (P > 0.25) from the initial young human open-eye edema recovery rate of 35.6 ± 3.4 μm/hr. Similarly, the initial rabbit closed-eye edema recovery rate of 14.3 ± 5.4 μm/hr was found to be not significantly different (P > 0.25) from the initial young human closed-eye edema recovery rate of 15.0 ± 2.2 μm/hr. Corneal edema recovery indices derived using nonlinear-regression analysis also had a strong similarity between rabbit and human corneal edema recovery rates. For example, the calculated percent recovery per hour (PRPH) for rabbit open-eye corneal edema recovery of 41.4 ± 11.3%/hr agreed well with the reported human open-eye PRPH of 34.2–58.9%/hr. The rabbit closed-eye PRPH of 25.6 ± 10.7%/hr was also found to lie between the calculated human closed-eye PRPH values of 19.9–30.2%/hr. The close similarity in both open-eye and closed-eye edema recovery values for human and rabbit corneas offers strong support for the use of the rabbit cornea as a model for human corneal edema recovery dynamics. Invest Ophthalmol Vis Sci 31:2003–2007, 1990

The rabbit cornea has been used extensively as a model in the study of corneal physiology.1–4 In particular, the rabbit has been commonly used for in vitro studies of corneal hydration control.5–8 The validity of the rabbit model has been questioned, however, after a report that the rabbit corneal endothelium, unlike that in humans, has a remarkable capacity to regenerate.9 A counterargument can be found in a report stating that the regenerative capacity of the rabbit endothelium is artifactual due to the use of immature animals.10,11 Thus, there is some uncertainty as to the validity of using the rabbit as a model for corneal hydration control studies.

Numerous reports propose that corneal hydration control can be assessed in vivo by measurement of corneal recovery from a particular level of stress-induced edema towards normal thickness.12–18 Two analysis methods were developed from these studies: (1) linear-regression analysis16–18 and (2) nonlinear-regression analysis.13,14 Linear-regression analysis estimates the initial rate of corneal edema recovery during the first stages of edema recovery. The usefulness of the linear-regression method is limited because it is unable to explain the nonlinear edema recovery curve seen experimentally. Nonlinear-regression analysis, on the other hand, offers a more realistic analysis model because it assumes that corneal deswelling follows an exponential path, whose shape may be determined by a small number of mathematical constants. This model is more clinically applicable; it can provide estimates of a number of corneal hydration parameters such as the corneal edema recovery rate, baseline corneal thickness, and the time required to recover to a particular fraction of the level of initial edema. Both of these models have been used to estimate corneal hydration control parameters in normal, aged, and diseased in vivo human corneas.12–15,18 No studies have as yet been done to assess the edema recovery characteristics of the in vivo rabbit cornea to our knowledge. We tried to quantify the open-eye and closed-eye edema recoveries of the in vivo rabbit cornea using both analysis methods. This study compared rabbit data with previously published human corneal edema recovery parameters and assessed the validity of the rabbit cornea as a model for recovery of the human corneal from induced edema.

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Materials and Methods

Subjects

Ten 19-week-old male New Zealand White rabbits were obtained from a local supplier (Ray Nichols, Lumberton, TX). The rabbits were housed in the Animal Care Unit of the University of Houston (AALAC approved) under a 12-hr light/12-hr dark cycle (lights on at 7 AM). Animals were allowed free access to food (Purina Rabbit Chow, Ralston-Purina, St. Louis, MO) and water, and were handled in accordance with the ARVO Resolution on the Use of Animals in Research.

Corneal Thickness Measurement

Central corneal thickness measurements were made by lightly touching the water-filled probe of an ultrasound pachometer (Storz Corneo-Scan 2000, St. Louis, MO) to the tear film of the rabbit cornea and obtaining six readings whose mean was used as the recorded corneal thickness measurement. No anesthetic was used, since the rabbits showed no discomfort during this procedure. The probe was hand-held and the position of the central cornea judged visually. This procedure has been previously shown to be valid, particularly so in the rabbit due to the relative constancy of the rabbit central corneal thickness. The ultrasound tissue velocity was set at 1580 m/sec.

Baseline Corneal Thickness

Because these laboratory rabbits tended to have irregular sleep patterns, baseline corneal thickness was determined by measuring the right eye corneal thickness of each rabbit every 2 hr for 24 hr. The average of these readings was used as an estimate of the baseline corneal thickness.

Edema Production

Patch test: Corneal edema was induced by patching both eyes closed for 2 hr. At the end of this time, the right eye was briefly opened, and the corneal thickness quickly measured. This procedure was done on all ten rabbits.

Lens test: Corneal edema was produced by inserting a thick soft hydrogel contact lens (+11.00B3; Bausch & Lomb, Rochester, NY) over the closed right eye for 2 hr. At the end of this time, the eye was briefly opened, the lens removed, and the corneal thickness quickly measured. This procedure was also done on all ten rabbits.

Edema Recovery

Open-eye: Open-eye edema recoveries were measured after both the Patch Test and Lens Test methods of producing edema. After inducing corneal edema, the eye was opened and the corneal thickness measured each 30 min for 3 hr (ie, seven measurements taken during the deswelling period). This procedure was done on all ten rabbits.

Closed-eye: Closed-eye edema recoveries were measured after both the Patch Test and Lens Test methods of producing edema. After inducing corneal edema, the eye remained closed and was opened briefly only during the measurement process. Corneal thickness measurements were taken hourly for 4 hr (ie, five measurements taken during the deswelling period). This procedure was also done on all ten rabbits.

Analysis Method

Linear-regression analysis: In the linear-regression analysis method, it is assumed that the initial phase of edema recovery can be modeled by a linear decrease of corneal thickness over time (μm/hr). The analysis was done during the first 1.5 hr of open-eye edema recovery and during the first 2 hr of closed-eye edema recovery. In both instances the correlation coefficient of the linear regression was found to be greater than 0.90.

Nonlinear-regression analysis: A mathematic model has been proposed for human open-eye corneal edema recovery. The model can be expressed as:

\[ T = B + S_i \exp^{-D \cdot t} + e \]

where \( T \) is the instantaneous corneal thickness (μm), \( B \) is the baseline corneal thickness (μm), \( S_i \) is the induced corneal swelling (μm), \( D \) is a calculated deswelling factor, \( t \) is the time after the start of the edema recovery (min), and \( e \) represents the error term of the mathematic model (ie, measurement error). This expression was shown to predict the open-eye edema recovery adequately in normal human subjects of various ages and in elderly subjects with Fuch's corneal endothelial dystrophy. The expression was also shown to provide clinical indices of corneal hydration control such as: (1) a corneal deswelling rate in percent recovery per hour (PRPH), (2) a time to remove 95% of the initial edema (T95%), and (3) the open-eye steady state corneal thickness (OESS). These edema recovery constants can be calculated by entering the raw corneal thickness data from the edema recovery experiments into a nonlinear-regression procedure (Marquardt method; SAS NLIN, Cary, NC). For a more detailed explanation, see Mandell et al and Poise et al.

Statistical analysis: Analysis of variance was used to test statistical differences between group means.
Results

Baseline Corneal Thickness

The mean rabbit corneal thickness over the 24-hr period was found to be 350 ± 10 \( \mu m \). This agrees well with literature values.\(^{22,23} \) All values reported here represent the mean of the experimental measurements ± one standard deviation.

Edema Production

Combining the data obtained from the open-eye and closed-eye procedures, it was found that the 2-hr insertion of the thick hydrogel contact lens (Lens Test) increased rabbit corneal thickness to 428 ± 16 \( \mu m \), producing 22% edema. Similarly, 2 hr of eye-closure (Patch Test) in the rabbit increased corneal thickness to 391 ± 16 \( \mu m \), producing 12% edema. The edema induced by the Lens Test was significantly greater (\( P < 0.0001 \)) than that induced by the Patch Test.

Edema Recovery

Open-eye edema recovery: The open-eye edema recovery functions from both the Lens Test and Patch Test are shown in Figure 1. The corneal thickness is seen to deswell in a nonlinear manner in both conditions until an asymptotic baseline is reached after approximately 3 hr at approximately 356 ± 11 \( \mu m \).

Closed-eye edema recovery: The closed-eye edema recovery functions from both the Lens Test and Patch Test are shown in Figure 2. The closed-eye recovery from the Lens Test underwent a nearly linear decrease in corneal thickness over 4 hr after the removal of the edema stimulus. In the Patch Test, the eye remained closed for 6 hr. The cornea was seen to reach maximal thickness 3-4 hr after eye closure; thereafter a relatively constant corneal thickness was attained.

Linear-regression analysis: The rate of open-eye edema recovery during the first hour after the Lens Test was calculated to be 36.3 ± 11.9 \( \mu m/hr \). The closed-eye edema recovery rate during the initial 2 hr of edema recovery after the Lens Test was calculated to be 14.3 ± 5.4 \( \mu m/hr \). These data are shown in Table 1.

Nonlinear-regression analysis: The test data were analyzed using the exponential open-eye edema recovery model.\(^{13,14} \) The calculated deswelling constants are listed in Table 2. After rejecting steady-state corneal thickness estimates with asymptotic standard errors greater than 5 \( \mu m \), the rabbit OESS corneal thickness was calculated to be 353 ± 14 \( \mu m \), in good agreement with the baseline estimate of corneal thickness of 350 ± 10 \( \mu m \). Due to the greater variability noted in the closed-eye edema recovery data, closed-eye steady state (CESS) estimates having asymptotic standard errors greater than 10 \( \mu m \) were rejected. These rejection criteria are more stringent than the 15-\( \mu m \) rejection criteria used by Polse et al.\(^{14} \) When this was done, the rabbit CESS corneal thick-

![Fig. 1. Normal rabbit open-eye edema recovery (n = 10). Error bars represent ±1 SEM. Measurements taken at 30-min intervals for 3 hr.](image)

![Fig. 2. Normal rabbit closed-eye edema recovery (n = 10). Error bars represent ±1 SEM. Measurements taken at 60-min intervals for 4 hr.](image)

Table 1. Corneal edema recovery rates in \( \mu m/hr \) (mean ± 1 SEM) derived using linear regression analysis using normal human data from the literature and experimental rabbit data (n = 10)

<table>
<thead>
<tr>
<th>Eye</th>
<th>Edema recovery (( \mu m/hr ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open Old human(^{17} )</td>
<td>26.5 ± 2.2</td>
</tr>
<tr>
<td>Rabbit</td>
<td>36.3 ± 3.8</td>
</tr>
<tr>
<td>Young human(^{17} )</td>
<td>35.6 ± 1.1</td>
</tr>
<tr>
<td>Closed Old human(^{17} )</td>
<td>10.5 ± 0.9</td>
</tr>
<tr>
<td>Rabbit</td>
<td>14.3 ± 1.7</td>
</tr>
<tr>
<td>Young human(^{17} )</td>
<td>15.0 ± 0.7</td>
</tr>
</tbody>
</table>

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Table 2. Corneal edema recovery constants (mean ± 1 SEM) derived using the exponential deswelling model using normal human data from the literature and experimental rabbit data (n = 10)

<table>
<thead>
<tr>
<th>Eye</th>
<th>PRPH (%/hr)</th>
<th>T95% (hr)</th>
<th>SS (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old human 14</td>
<td>34.2 ± 2.3</td>
<td>7.5 ± 0.6</td>
<td>537 ± 10</td>
</tr>
<tr>
<td>Normal rabbit</td>
<td>41.4 ± 3.6</td>
<td>6.2 ± 0.8</td>
<td>353 ± 4</td>
</tr>
<tr>
<td>Young human 14</td>
<td>58.9 ± 2.8</td>
<td>3.5 ± 0.3</td>
<td>516 ± 12</td>
</tr>
<tr>
<td>Closed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Old human 17</td>
<td>19.9</td>
<td>13.3</td>
<td>—</td>
</tr>
<tr>
<td>Normal rabbit</td>
<td>25.6 ± 3.4</td>
<td>12.6 ± 2.3</td>
<td>388 ± 4</td>
</tr>
<tr>
<td>Young human 17</td>
<td>30.2</td>
<td>8.3</td>
<td>—</td>
</tr>
</tbody>
</table>

Discussion

A major factor in the use of an animal model is its applicability to the human condition. Figure 3 illustrates the open-eye edema recoveries for rabbit and human corneas. The close similarity of the rabbit and human open-eye edema recovery functions support the proposal that the rabbit may provide a useful model for corneal deswelling dynamics. Further support may be obtained by considering the results of the mathematic analysis of the edema recovery data.

Using linear-regression analysis, for the first hour, the young human (mean age, 27 yr) and rabbit open-eye edema recoveries were found to be similar (P > 0.25) at 35.6 ± 3.4 µm/hr and 36.3 ± 11.9 µm/hr. The closed-eye edema recoveries for young human and rabbit corneas, for the initial 2 hr of deswelling, were also similar (P > 0.25) at 15.0 ± 2.2 µm/hr and 14.3 ± 5.4 µm/hr.

Further data supporting the similarity between human and rabbit corneal deswelling rates can be found by comparing the linear-regression open-eye and closed-eye deswelling rates found for the rabbit with human data from varying age groups. These data are shown in Table 1. The rabbit open-eye deswelling rate fell near the value reported for the young (mean, 27 yr) human and the old (mean, 66 yr) human. Similarly, the rabbit closed-eye deswelling rate was found to lie between the young human and old human deswelling rates. These similarities, as found by linear-regression analysis, further strengthen the use of the rabbit cornea in studies of corneal edema recovery dynamics.

The human and rabbit corneal edema recovery constants calculated using nonlinear-regression analysis are listed in Table 2. It can be seen that the open-eye and closed-eye edema recovery constants for the rabbit cornea agree closely with human values, with the rabbit constants lying between those reported for old (mean, 72 yr) and young (mean, 24 yr) human subjects. This result, when combined with the previous results, offers strong support for the use of the rabbit model for studies of corneal edema recovery.

On a clinical level, our investigation supports the claims of Mandell et al and Poise et al that the exponential edema recovery model may enable clinical assessment of in vivo corneal hydration control. With the current availability of fast and reliable ultrasound pachometers, we can foresee immediate clinical applications in contact lens wear and pathology detection. Unfortunately, and in agreement with Mandell et al and Poise et al, a great deal of refinement is required before this test can become a clinically useful tool. At present, the long testing durations and computer-intensive analysis techniques restrict this method to a laboratory setting.

In conclusion, we provided evidence to support the use of the human open-eye exponential edema recovery model for the analysis of human closed-eye corneal edema recovery. The model has also been shown to be useful in describing both the open-eye and
and closed-eye edema recovery in rabbit cornea. Although an extremely powerful laboratory tool for assessment of in vivo corneal hydration control, a great deal of refinement of the analysis techniques and experimental design is necessary before this method may be used clinically. The close similarities between human and rabbit corneal edema recoveries, using both linear- and nonlinear-regression analysis, strongly suggest that the rabbit cornea offers a useful model for the study of corneal edema recovery dynamics.

Key words: cornea, deswelling, pachometry, rabbit

Acknowledgments

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References