Effects of Topical H-7 on Outflow Facility, Intraocular Pressure, and Corneal Thickness in Monkeys

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Objectives: To determine if low concentrations of H-7 (1-[5-isoquinoline sulfonyl]-2-methyl piperazine) topically applied to the eye increases outflow facility and decreases intraocular pressure (IOP) without affecting the cornea in monkeys, and to evaluate if the effect of H-7 on IOP is pressure dependent.

Methods: Single or multiple doses of 5% H-7 or vehicle (20 µL) were administered topically to opposite eyes of normal monkeys. A single dose of 2% H-7 or vehicle (50 µL) was administered to the glaucomatous eye of monkeys with laser-induced unilateral glaucoma, with vehicle on day 1 and H-7 on day 2.

Results: In normotensive eyes, 1 dose of 5% H-7 maximally decreased IOP by a mean±SEM of 2.5±1.0 mm Hg (−16.7%±5.5%) at 3 hours. Higher baseline IOP and repeated dosing were associated with greater IOP reduction. Outflow facility was increased, but central corneal thickness was not affected. In glaucomatous eyes, 1 dose of 2% H-7 maximally decreased IOP by a mean±SEM of 5.8±0.6 mm Hg (−16.9%±1.6%) at 2 hours.

Conclusions: Five percent H-7 increases outflow facility and decreases IOP, but does not affect corneal thickness. Multiple doses of H-7 induce greater reduction of IOP than a single dose. The effect of H-7 on IOP may be pressure dependent.

Clinical Relevance: Multiple topical treatments with low doses of H-7 or analogues may substantially reduce outflow resistance in the hypertensive eye without meaningfully affecting the cornea.

**Table 1. Protocol Description**

<table>
<thead>
<tr>
<th>Group</th>
<th>Before Day 1</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (n = 7) 20 µL (4 × 5 µL) of 5% H-7 and vehicle (isotonic sodium chloride) administered topically in opposite eyes</td>
<td>BL pachymetry under ketamine 3 or 5 d before the first dose</td>
<td>Tonometry at 0 h and hourly from 1-6 h after the first dose, and then the second dose at 4 PM under ketamine</td>
<td>The third and fourth doses at 8 AM and 4 PM under ketamine</td>
<td>Pachymetry before and 1-6 h after the fifth dose under ketamine (n = 6 because 1 monkey was deleted from pachymetry due to cornea edema)</td>
<td>NA</td>
</tr>
<tr>
<td>2 (n = 6) 20 µL of 5% H-7 and vehicle (isotonic sodium chloride) administered topically in opposite eyes</td>
<td>NA</td>
<td>BL tonometry and then the first dose (4 × 5 µL) under ketamine at about 8 AM. The second dose at 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>The third and fourth doses at 8 AM and 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>The fifth and sixth doses at 8 AM and 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>Tonometry at 0 h and hourly from 1-6 h after the seventh dose (4 × 5 µL)</td>
</tr>
<tr>
<td>3 (n = 6) 20 µL of 5% H-7 and vehicle (isotonic sodium chloride with 25% dimethyl sulfoxide) administered topically in opposite eyes</td>
<td>NA</td>
<td>The first and second doses at 8 AM and 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>The third and fourth doses at 8 AM and 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>The fifth and sixth doses at 8 AM and 4 PM (2 × 10 µL) to fully conscious and manually restrained monkeys</td>
<td>Perfusion immediately before and 1½ h after the seventh treatment (4 × 5 µL under pentobarbital)</td>
</tr>
<tr>
<td>4 (n = 6†) 20 µL (4 × 5 µL) of 5% H-7 and vehicle (isotonic sodium chloride) administered topically in opposite eyes</td>
<td>Screening for monkeys with higher BL IOP (≥16 mm Hg)</td>
<td>Tonometry at 0 h and hourly from 1-6 h after a single dose under ketamine</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>5 (n = 6) Glaucomatous monkeys with unilateral laser-induced ocular hypertension</td>
<td>NA</td>
<td>BL tonometry at 0 h and hourly 1-6 h after 1 dose of isotonic sodium chloride (2 × 25 µL) topically to the ocular hypertensive eye at 9:30 AM</td>
<td>Tonometry at 0 h and hourly 1-6 h after 1 dose of 2% H-7 (2 × 25 µL) topically to the same hypertensive eye at 9:30 AM</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: BL, baseline; NA, not available. †Two monkeys are from group 1.

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**METHODS**

**ANIMALS AND ANESTHESIA**

Thirty-two adult cynomolgus monkeys (*Macaca fascicularis*) of both sexes, weighing 2 to 5 kg, were involved in this study. All experiments were conducted in accord with the University of Wisconsin–Madison Medical School and Mount Sinai School of Medicine institutional animal care and use committees, with the National Institutes of Health guidelines, and with the Association for Research in Vision and Ophthalmology Statement on the Use of Animals in Ophthalmic and Vision Research. Five percent H-7 was used in 24 normotensive monkeys studied at the University of Wisconsin–Madison Medical School as 4 groups (groups 1–4); 3 monkeys were included in both group 1 and group 3, and 2 monkeys were included in both group 1 and group 4. Monkeys in group 3 had undergone prior anterior chamber (AC) perfusions, but not within the preceding 5 to 6 weeks. Monkeys in group 4 had higher baseline IOP (mean IOP, 16 mm Hg) under ketamine hydrochloride (hereafter ketamine) anesthesia in both eyes compared with most of the normal cynomolgus monkeys. Two percent H-7 was used in 8 monkeys with unilateral ocular hypertension (mean IOP, 30-35 mm Hg) induced by repeated argon or diode laser photocoagulation of the TM11 studied at the Mount Sinai School of Medicine as group 5. The glaucomatous monkeys had not received any treatment for at least 2 weeks before the testing. All monkeys were free of aqueous humor cells and flare as assessed by slitlamp biomicroscopy. In normal monkeys, anesthesia for tonometry or pachymetry was induced with intramuscular ketamine (10 mg per kilogram of body weight) and maintained with supplemental intramuscular injections as required (usually 5 mg/kg every 30-45 minutes). Anesthesia for AC perfusion was induced with intramuscular ketamine (10 mg/kg), followed by intravenous pentobarbital sodium (15 mg/kg). In glaucomatous monkeys, anesthesia for tonometry was induced with intramuscular ketamine (2-5 mg/kg), with 1 drop of 0.5% proparacaine hydrochloride applied topically 5 minutes before tonometry.

**DRUG PREPARATION AND ADMINISTRATION**

H-7 was obtained from Sigma-Aldrich Corporation (St Louis, Mo). Five percent H-7 was freshly dissolved for topical administration in isotonic sodium chloride alone or with 25% dimethyl sulfoxide (1 mg/20 µL); isotonic sodium chloride with (group 3) or without (groups 1, 2, and 4) 25% dimethyl sulfoxide served as a vehicle control. The pH (tested by pH paper) of 5% H-7 in isotonic sodium chloride was approximately 2 to 3 (adjustment of pH with sodium hydroxide reduces solubility of H-7). To increase the pH of 5% H-7, we dissolved the drug in 25% dimethyl sulfoxide with isotonic sodium chloride in group 3.
Twenty-five percent dimethyl sulfoxide seemed to improve the solubility of H-7 when the pH was slightly elevated (eg, approximately 3-4). Two percent H-7 was freshly prepared in isotonic sodium chloride (1 mg/50 μl), and isotonic sodium chloride served as a vehicle control. Administrations of H-7 or vehicle in the different groups are shown in Table 1. For normal monkeys, H-7 or vehicle (4 × 5 μl) was administered topically to the central cornea of the supine monkey under ketamine anesthesia for tonometry and pachymetry or was administered topically to the superior cornea of the prone monkey under pentobarbital sodium for perfusion. To reduce any potential cumulative effect of repeated ketamine anesthesia on IOP or outflow facility during the multiple treatments, some doses of H-7 or vehicle were administered topically to fully conscious and manually restrained monkeys, with fewer and larger drops (2 × 10 μl) applied because of the limited cooperative period from conscious monkeys. For glaucomatous monkeys, H-7 or vehicle (2 × 25 μl) was administered topically to the central cornea in a manner similar to that in ketamine-anesthetized normal monkeys.

### IOP Measurement

In normal monkeys, IOP was determined with a minified Goldmann applanation tonometer, using fluorescein as the tear film indicator, with the monkey lying prone in a head holder. For each eye, 3 IOP readings were averaged as a baseline before administration of H-7 or vehicle, and single IOP readings were taken after the drug or vehicle administration at different time points (Table 1). In glaucomatous monkeys, IOP was determined with a calibrated pneumatonometer (model 30 classic; Mentor Inc, Norwell, Mass).

### Outflow Facility and Outflow Rate Measurement

Total outflow facility was determined in normotensive monkeys of group 3 by 2-level constant pressure (approximately 15 or 25 mm Hg) perfusion of the AC with Bârany mock aqueous humor, using a 1-needle technique and correcting for internal apparatus resistance (Table 1 and Table 2).

### Corneal Thickness Measurement

Central corneal thickness was determined in normal monkeys (group 1) by ultrasonic pachymetry (DGH-1000 ultrasonic pachymeter; DGH Technology, Inc, Solana Beach, Calif). For each eye, 3 readings taken 3 or 5 days before the first dose of 5% H-7 or vehicle were averaged as a baseline. A single value was taken before the fifth treatment; after the fifth treatment, measurements were taken every 30 minutes for 4 hours and then hourly for 2 hours.

### Slitlamp Examination

In normal monkeys, slitlamp biomicroscopy was performed before drug administration, during IOP measurement (1, 3, and 6 hours after drug administration), and before pachymetry and AC perfusion. In glaucomatous monkeys, slitlamp examination was performed before and 1, 3, and 5 hours after drug administration. The integrity of the corneal epithelium and endothelium, the presence of flare or cells in the AC, and the clarity of lenses were noted. All normal animals were free of preexisting ocular abnormalities when studied. All glaucomatous monkeys were free of aqueous humor flare and cells when studied.

### Data Analysis

Data are given as mean ± SEM for the number of eyes or animals. In normal monkeys (groups 1-4), comparisons were made using the 2-tailed paired t test for differences vs 0.0 or for ratios vs 1.0 (predrug or postdrug treated vs control treated, postdrug or postvehicle vs ipsilateral baseline, and baseline-corrected postdrug treated vs control). In glaucomatous monkeys (group 5), IOP difference between day 1 (vehicle) and day 2 (H-7) in the ocular hypertensive eye was tested by the 2-tailed paired t test.

### Results

#### Intraocular Pressure

Intraocular pressure changes from 1 to 6 hours after H-7 or vehicle in the different groups are shown in Figures 1, 2, and 3. Maximal IOP reductions after H-7 in the different groups are shown in Table 3.

#### Outflow Facility and Outflow Rate

In group 3 (normal monkeys), H-7 significantly increased outflow facility by 77% ± 32% (n=8, P<.05) during the overall 90-minute perfusion beginning 1 ½ hours after the seventh dose, adjusted for outflow facility before the seventh dose (baseline) and contralateral control eye washout (Table 2). Outflow rate at 25 mm Hg during baseline or postdrug measurement was higher than that at 15 mm Hg in the H-7–treated eye and the contralateral control eye. The increase of outflow rate in the H-7–treated eye at 25 mm Hg was similar to that at 15 mm Hg, after adjustment for baseline and contralateral control eye washout.
CORNEAL THICKNESS

Central corneal thicknesses at baseline and before the fifth treatment were 456.3±15.6 and 469.8±15.7 µm, respectively, in the H-7–treated eye and 449.7±14.8 and 456.2±15.1 µm, respectively, in the contralateral control eye. The central corneal thickness after the fifth treatment varied between 455.8±17.9 and 471.0±15.6 µm in the H-7–treated eye and between 449.2±13.2 and 461.7±14.4 µm in the contralateral control eye during 6-hour pachymetry. No significant difference in the central corneal thickness between eyes was observed at any indicated time (P>.60), after adjustment for ipsilateral baseline (Figure 4).

SLITLAMP EXAMINATION

During IOP measurement in normal monkeys, most monkeys in groups 1, 2, and 4 had mild punctate corneal epithelial defects (PCEDs) in both eyes, but the defects in H-7–treated eyes were slightly more apparent than in control eyes (Table 4). Two monkeys (one in group 1 and another in group 2) that had shown continuous nystagmus under ketamine anesthesia during IOP measurement had a small epithelial defect in the central cornea of the H-7–treated eye at the end of tonometry. The monkey in group 2, but not the one in group 1, had corneal cloudiness and edema 2 days later. Another monkey in group 1 that had shown some PCEDs during tonometry on day 1 had a central corneal epithelial defect before the third treatment on day 2 (presumably occurring in the cage) and corneal cloudiness and edema on day 3. However, the cornea was normal for all monkeys approximately 16 hours after the sixth dose of H-7 in group 2 (before tonometry) and group 3 (before AC perfusion). In addition, the PCEDs seen during tonometry earlier had

Figure 1. Effect of 5% topical H-7 on intraocular pressure (IOP). A, One dose (group 1, n=7). B, Seven doses (group 2, n=8). Intraocular pressure data are given as mean±SEM millimeters of mercury for the number of animals. Intraocular pressure difference between eyes corrected for baseline was tested for differences vs 0.0 by the 2-tailed paired t test: * indicates P<.05; †, P<.03; ‡, P<.02; §, P<.01; ||, P<.005; and ¶, P<.001.

Figure 2. Effect of a single dose of 5% H-7 on intraocular pressure (IOP) in monkeys with different baseline (BL) IOP. A, Monkeys with lower BL IOP (group 1b, n=5; Table 3). B, Monkeys with higher BL IOP (group 4, n=6). Intraocular pressure data are given as mean±SEM millimeters of mercury for the number of animals. Intraocular pressure difference between eyes corrected for baseline was tested for differences vs 0.0 by the 2-tailed paired t test: * indicates P<.03; †, P<.01; ‡, P<.005; and dashed line, 16 mm Hg.
disappeared or significantly decreased in both eyes of most monkeys approximately 16 hours after the fourth dose (before pachymetry) in group 1. No other abnormality was observed in any monkey in any protocol during slitlamp examination in groups 1, 2, and 4. In glaucomatous monkeys (group 5), 1 of 8 ocular hypertensive eyes had mild PCEs and 5 hours after drug administration.

This study showed that single and multiple doses of 5% H-7 decrease IOP in normotensive monkeys, with multiple doses producing greater IOP reduction. This additive effect of multiple treatments occurs with many antiglaucoma drugs in normotensive\textsuperscript{5,10} and glaucomatous\textsuperscript{5,7,18} monkeys. In the present study, IOP reduction after H-7 in normal monkeys with higher baseline IOP (mean IOP, 17 mm Hg) was greater than that in monkeys with lower baseline IOP (mean IOP, approximately 13 mm Hg), consistent with higher outflow rate at 25 mm Hg than at 15 mm Hg during perfusion. This, in conjunction with previous findings,\textsuperscript{5,9,10} suggests that H-7 decreases IOP by reducing outflow resistance in the TM and that the effect of H-7 on outflow resistance is pressure dependent. The similarity of outflow rates as defined by the double ratios ([H-7/Baseline] / [Vehicle/Baseline]) at 25 and 15 mm Hg indicates that the perfusion-induced resistance washout during bilateral baseline and contralateral postvehicle measurements is also pressure dependent. One can reasonably hypothesize that still lower concentrations of H-7 in the hypertensive eye may show the same or stronger effect as 5% H-7 does in normotensive monkeys. As previously hypothesized,\textsuperscript{10} lower concentrations and total doses in higher volumes might minimize or avoid corneal toxicity induced by high concentrations of H-7.

To test these hypotheses, a single dose of 50 µL of 2% H-7 was applied in laser-induced hypertensive eyes. Fifty microliters of 2% H-7 in glaucomatous eyes produced a similar IOP reduction compared with 20 µL of 5% H-7 in the normotensive eyes of group 1 (−16.9% vs −16.7%). However, compared with the normotensive eyes of group 4, which had higher baseline IOP, the maximal IOP reduction in the glaucomatous eyes after 50 µL of 2% H-7 was smaller than that in the normal eyes after 20 µL of 5% H-7 (−16.9% vs −24.8%). Although 50 µL of 2% H-7 contains the same amount of H-7 (1 mg) as 20 µL of 5% H-7, the former may be less effective than the latter when administered topically in the monkey eye because the 50-µL volume far exceeds the capacity of the monkey’s cul-de-sac, so that some of the drug may overflow during the application. This may reduce the amount

![Graph](https://via.placeholder.com/150)

**Figure 3.** Effect of a single dose of 2% H-7 on intraocular pressure (IOP) in the laser-induced unilateral glaucomatous monkey eyes (group 5, n=8). IOP data are given as mean±SEM millimeters of mercury for the number of animals (eyes). IOP was measured in the eye before and after H-7 on day 2; *P<.05; †, P<.005; and ‡, P<.001.

<table>
<thead>
<tr>
<th>Group</th>
<th>BL, mm Hg</th>
<th>Time, h</th>
<th>Difference, mm Hg</th>
<th>%</th>
<th>BL, mm Hg</th>
<th>Time, h</th>
<th>Difference, mm Hg</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a (n=7)</td>
<td>13.6 ± 0.8</td>
<td>3</td>
<td>2.5 ± 1.0†</td>
<td>16.7 ± 5.5†</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>1b (n=5)</td>
<td>12.6 ± 0.6</td>
<td>4</td>
<td>1.53 ± 0.6</td>
<td>14.2 ± 4.9‡</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2 (n=6)</td>
<td>...</td>
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<td>...</td>
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<td>...</td>
<td>...</td>
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</tr>
<tr>
<td>4 (n=6)</td>
<td>16.9 ± 0.4</td>
<td>2</td>
<td>4.0 ± 0.8§</td>
<td>24.8 ± 5.2§</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>5 (n=6)</td>
<td>34.1 ± 2.0</td>
<td>6</td>
<td>5.8 ± 0.6</td>
<td>16.9 ± 1.6</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SEM for the number of animals, each contributing 1 H-7−treated eye and 1 vehicle-treated eye in normotensive monkeys, or each contributing 1 laser-induced hypertensive eye that received vehicle on day 1 and H-7 on day 2 unless otherwise indicated. Intracocular pressure was measured from 1 to 6 hours after single or multiple doses of H-7 in normotensive monkeys (groups 1, 2, and 4) or after a single dose of 2% topical H-7 in glaucomatous monkeys (group 5). Baseline IOP (BL) was measured immediately before the first dose in groups 1, 2, and 4, or from 1 to 6 hours after vehicle the day before H-7 treatment in group 5. Group 1a included all 7 monkeys selected randomly; group 1b excluded 2 monkeys from group 1a that had higher BL (≥16 mm Hg); the 2 excluded monkeys were added into group 4, in which all monkeys had BL of 16 mm Hg and above. Data are the maximal IOP reduction at the indicated time (hours after H-7) after single or multiple doses of H-7. In groups 1, 2, and 4, Difference indicates [H-7−BL] / [Vehicle−BL]; and Percentage, (1 − [H-7/Baseline] / [Vehicle/Baseline]) × 100; and in group 5, Difference indicates H-7 − Vehicle; and Percentage, (1 − H-7/Vehicle) × 100; where H-7 indicates IOP in H-7−treated eye; and Vehicle, IOP in vehicle-treated eye. Intracocular pressure differences between eyes were tested by the 2-tailed paired \( t \) test. Ellipses indicate not available.

†, P<.05.
‡, P<.01.
§, P<.005.
||, P<.001.
of drug penetrating into the eye and, in turn, the concentration of the drug in the AC. This speculation is in agreement with previous studies in which the bioavailability of topical medications can be increased by decreasing the volume of eyedrops. In addition, laser-induced structural changes in outflow pathway may cause the absence of an expected baseline pressure-dependent IOP reduction in the glaucomatous eye. Based on previous studies, laser photocoagulation of the TM induces scar tissue formation and decreases normal cells in the outflow pathway, which increases the tissue’s stiffness and limits its relaxation after H-7. Therefore, although most clinical and investigational ocular hypertensive agents are more effective in the laser glaucomatous monkey model than in the normotensive monkey eye, it may not be so for cytoskeletal drugs (eg, H-7) that increase outflow facility by inhibiting cellular contractility in the TM and inner wall of Schlemm canal. Given the fact that 5% H-7 produced greater IOP reduction in group 4 than in group 1, H-7 might produce greater ocu-

Table 4. Results of Punctate Corneal Epithelial Defects (PCEDES) by Slitlamp Examination*

<table>
<thead>
<tr>
<th>Group</th>
<th>Doses</th>
<th>Time After Dose, h</th>
<th>After H-7</th>
<th>After Vehicle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td>1</td>
<td>Tonometry (n = 7)</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Pachymetry (n = 6)</td>
<td>4</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>16</td>
<td>8</td>
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<tr>
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<td></td>
<td></td>
<td>5</td>
<td>7</td>
<td>1</td>
</tr>
</tbody>
</table>

*Data are given as number of eyes of the number of animals.
†On a scale of 0+ to 4+, where 0 indicates essentially clear; 1+, densely packed PCEDES covering one quarter or less of the corneal surface or diffusely packed PCEDES distributed over the entire cornea; 2+, densely packed PCEDES covering one quarter to one half of the corneal surface or moderately packed PCEDES distributed over the entire cornea; 3+, densely packed PCEDES covering one half to three quarters of the corneal surface or moderately densely packed PCEDES distributed over the entire cornea; and 4+, densely packed PCEDES covering three quarters to all of the corneal surface.
lar hypotension in non–laser-induced glaucoma models in the monkey eye (eg, steroid-induced glaucoma27-29) or in glaucomatous human eyes in which anatomy is relatively undisturbed. Further studies are needed to clarify this issue.

Unlike a single topical dose of approximately 15% H-7 (2.9 mg/20 µL),10 5 doses of 5% H-7 (1 mg/20 µL) given topically did not significantly increase the central corneal thickness. This indicates that the 5% concentration of the drug does not significantly affect the corneal endothelium. As assessed by slitlamp biomicroscopy, 5% H-7 is also less toxic to the corneal epithelium.19 Mild PCDs in both eyes are a common phenomenon during tonometry in ketamine-anesthetized animals and may be due to reduced blinking under ketamine anesthesia and frequent IOP measurements. However, compared with the contralateral control eyes, the slightly more apparent PCDs in the H-7-treated eyes indicate that 5% H-7 may still mildly affect the corneal epithelium in living monkey eyes. Two monkeys that had a central corneal epithelial defect during tonometry had continuous vertical nystagmus after ketamine anesthesia. This suggests that 5% H-7 could render the cornea more susceptible to mechanical damage (eg, frequent contact by the moving eye with the tip of the Goldmann applanation tonometer). Similarly, touching the eye by hand because of an uncomfortable feeling following drug treatment and tonometry may be responsible for one monkey's developing a central corneal epithelial defect in its cage. However, this study shows that multiple doses of 5% H-7 seem to have no effect on the cornea as seen by slitlamp examination when the cornea is untouched. In addition, a single dose of 2% H-7 (1 mg/50 µL) did not apparently affect the corneal epithelium as assessed by slitlamp examination in glaucomatous monkeys, compared with a single dose of 5% H-7 (1 mg/20 µL) in normal monkeys. This seems to support the hypothesis from a previous study10 that repetitive lower concentrations and total doses in higher volumes, spread out over the entire corneal or conjunctival surface, may minimize or avoid corneal toxicity.

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