Effects of Netarsudil on the Corneal Endothelium

Three-Month Findings from a Phase 3 Trial

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Purpose: To describe the changes in endothelial cell density (ECD), the coefficient of variation (CV), and the percent of hexagonal cells (%HEX) after 3 months of therapy with netarsudil (Rhopressa; Aerie Pharmaceuticals Inc, Durham, NC) 0.02% dosed once daily (QD) or twice daily (BID) and to compare these changes with those seen with timolol 0.5% BID in eyes with ocular hypertension (OHTN) or open-angle glaucoma (OAG).

Design: Post hoc analysis of data from a phase 3 evaluation of the intraocular pressure (IOP)-lowering efficacy and safety of netarsudil 0.02% versus timolol 0.5%.

Participants: A subset of study subjects underwent corneal endothelial cell imaging by specular microscopy at baseline and after 3 months of therapy.

Methods: Images were evaluated in a masked fashion at an independent reading center. The ECD, CV, and %HEX were determined using a standardized protocol for image analysis.

Main Outcome Measures: Changes in ECD, CV, and %HEX from baseline to 3 months were compared between treatment groups using 2-sample t tests.

Results: Data from 386 subjects from whom analyzable specular microscopy images were obtained at both baseline and month 3 were included in this analysis. Mean ECD, CV, and %HEX values were comparable between groups at baseline. There were no statistically significant between-group differences in changes from baseline to month 3 in ECD, CV, or %HEX between either of the netarsudil groups and the timolol group. Within groups, CV declined in a statistically significant fashion from baseline to month 3 in all 3 groups by 1.4% to 2.1% (P < 0.001), and %HEX increased by a statistically significant amount (0.7%, P = 0.030) in the timolol group. These small changes were unlikely to be of clinical significance. No statistically significant changes in ECD were seen in any group.

Conclusions: Netarsudil 0.02% showed no clinically significant effects on ECD, CV, or %HEX when dosed QD or BID for 3 months in eyes with OHTN or OAG. Ophthalmology Glaucoma 2020;3:421-425 © 2020 by the American Academy of Ophthalmology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

See Editorial on page 407.

Netarsudil ophthalmic solution 0.02% (Rhopressa [Aerie Pharmaceuticals Inc, Durham, NC]; netarsudil 0.02%), a Rho kinase (ROCK) inhibitor, was approved in December 2017 by the US Food and Drug Administration for the reduction of elevated intraocular pressure (IOP) in eyes with open-angle glaucoma (OAG) or ocular hypertension (OHTN). In the Rho Kinase Elevated IOP Treatment Trials 1 and 2 (ROCKET-1 and ROCKET-2) phase 3 clinical trials, netarsudil provided a 3.3- to 5.0-mmHg IOP reduction across 9 time points over 3 months, with common adverse events that included conjunctival hyperemia, corneal verticillata, and conjunctival hemorrhages. Three mechanisms of action have been proposed for the effect of netarsudil on IOP, including an increase in trabecular outflow, a reduction of aqueous formation, and a reduction of episcleral venous pressure. The recent withdrawal of the CyPass supraciliary micro-stent (Alcon Laboratories, Inc, Fort Worth, TX) due to late reductions in endothelial cell density (ECD) has drawn attention to the complex relationship between the corneal endothelium and glaucoma and its treatment. The ECD declines steadily with age, and this loss can be accelerated in eyes with glaucoma by factors intrinsic to the disease itself (increased IOP, pseudoexfoliation, angle closure) or by therapies that reduce IOP, including laser and incisional procedures such as trabeculectomy, tube-shunt implantation, and minimally invasive glaucoma surgeries. However, the landmark Ocular Hypertension Treatment Study (OHTS) found no relationship between changes in ECD and the use of topical IOP-lowering medical therapy.
In the ROCKET-2 study, specular microscopy was performed at study centers with the appropriate equipment onsite. In this analysis, changes in ECD and corneal endothelial cell morphology (the coefficient of variation [CV] and the percent of hexagonal cells [%HEX]) seen in the ROCKET-2 study are described.

**Methods**

The ROCKET-2 was a prospective, multicenter, double-masked, randomized, active-controlled, parallel-group, phase 3 clinical trial that evaluated the efficacy and safety of netarsudil 0.02% once daily (QD) and netarsudil 0.02% twice daily (BID) compared with timolol 0.5% BID for 12 months, with an interim analysis conducted at month 3. Details of the study design have been described previously. The study was conducted in full accordance with the Declaration of Helsinki and the Health Information Portability and Accountability Act. The study protocol was reviewed and approved by all relevant ethics boards. All participants provided informed consent in writing. The study was registered at clinicaltrials.org (NCT02207621) before the enrollment of any subjects.

Eligibility criteria for ROCKET-2 have been previously described. Briefly, eligible subjects were adults (≥18 years of age) or children aged 0 to 2 years with bilateral OAG or OHTN, with unmedicated IOP in at least 1 eye >20 mmHg and <27 mmHg at 8:00 AM on 2 qualification visits and IOP >17 mmHg and <27 mmHg at 10:00 AM and 4:00 PM on the second qualification visit. If both eyes qualified for all criteria, the eye with higher IOP was selected to be the study eye. Key exclusion criteria included use of more than 2 IOP-lowering medications at screening, presence of pseudoexfoliation or pigment dispersion syndrome, narrow angles or a history of angle closure, or prior glaucoma surgery. Key exclusion criteria specifically related to corneal health included central corneal thickness >600 μm, current active herpes simplex or zoster keratitis, clinically significant corneal edema, severe keratoconjunctivitis sicca, or any ocular pathology that would prevent accurate assessment of IOP by applanation tonometry. Specular microscopy was not a required assessment in this study (i.e., lack of specular microscopy was not considered a protocol deviation), and thus there were no eligibility criteria specific to baseline ECD or endothelial cell morphology.

Eligible participants were randomized to receive netarsudil 0.02% QD (with QD placebo vehicle for masking) or BID, or timolol 0.5% BID. The schedule of visits included a screening evaluation, 2 qualification visits after washout of IOP-lowering medications if applicable (the latter of which served as the baseline visit and first day of dosing), and 3 on-treatment visits at week 2, week 6, and month 3. At the baseline visit and each of the 3 on-treatment visits, IOP was measured at 8:00 AM, 10:00 AM, and 4:00 PM. At baseline and the month 3 visit, 3 images of the central corneal endothelium were obtained by Konan specular microscopy at the 42 participating sites at which specular microscopy was available; all subjects enrolled at these sites were included in this analysis. After calibration of the instrument and certifying on image quality, these images were evaluated in a masked fashion at an independent reading center (Cornea Image Analysis Reading Center, Case Western Reserve University and University Hospitals Cleveland Medical Center, Cleveland, OH). Endothelial cell density, CV, and %HEX were determined using a standardized protocol for image analysis.

The primary statistical objective of this post hoc analysis was to characterize the changes in ECD and endothelial cell morphology observed between baseline and month 3 and to compare these changes between treatment groups. Differences in demographics between groups were evaluated using Fisher exact tests for categorical variables and 1-way analysis of variance for continuous variables. Changes from baseline in ECD, CV, and %HEX within groups were evaluated using paired t tests, whereas comparisons between groups were based on 2-sample t tests. All tests were 2-sided, and P < 0.05 was taken as the level of significance. The study was powered, and sample size determined, for its overall primary objective (IOP reduction) and not for safety analyses.

**Results**

Overall, 756 subjects in ROCKET-2 were randomized to treatment with netarsudil 0.02% QD (n = 251), netarsudil 0.02% BID (n = 254), or timolol 0.5% BID (n = 251) at 62 study sites. Of these, 386 subjects enrolled at 42 sites who underwent analyzable specular microscopy at both baseline and month 3, and whose month 3 visit was within the protocol-specified window (±3 days) were evaluated (134 [53.4%], 96 [37.8%], and 156 [62.2%] subjects, respectively). Demographic data for this corneal safety cohort are given in Table 1. Briefly, their mean (standard deviation) age was 64.5 (12.4) years, 69.2% were white, 29.0% were black, and 57.3% were female; there were no significant between-group differences in any demographic factors.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Netarsudil 0.02% QD</th>
<th>Netarsudil 0.02% BID</th>
<th>Timolol 0.5% BID</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study eye diagnosis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OHTN</td>
<td>47 (35.1)</td>
<td>27 (28.1)</td>
<td>44 (28.2)</td>
</tr>
<tr>
<td>OAG</td>
<td>87 (64.9)</td>
<td>69 (71.9)</td>
<td>112 (71.8)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>75 (56.0)</td>
<td>55 (57.3)</td>
<td>91 (58.3)</td>
</tr>
<tr>
<td>Male</td>
<td>59 (44.0)</td>
<td>41 (42.7)</td>
<td>65 (41.7)</td>
</tr>
<tr>
<td>Age (yrs), mean (SD)</td>
<td>64.9 (11.7)</td>
<td>65.4 (13.5)</td>
<td>63.7 (12.3)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>95 (70.9)</td>
<td>64 (66.7)</td>
<td>108 (69.2)</td>
</tr>
<tr>
<td>Black or African American</td>
<td>35 (26.1)</td>
<td>32 (33.3)</td>
<td>45 (28.8)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (3.0)</td>
<td>0 (0)</td>
<td>3 (1.9)</td>
</tr>
</tbody>
</table>

BID = twice daily; OAG = open-angle glaucoma; OHTN = ocular hypertension; QD = once daily; SD = standard deviation.

*Differences in demographic parameters were not statistically significant among treatment groups.*
Baseline and month 3 ECD, CV, and %HEX data are given in Table 2. Mean corneal ECD in the study eye of patients in the netarsudil QD, netarsudil BID, and timolol groups was 2482, 2450, and 2456 cells/mm², respectively, at baseline and 2482, 2468, and 2445 cells/mm², respectively, at month 3. Mean CV in the study eye of patients in the netarsudil QD, netarsudil BID, and timolol groups was 32.5%, 32.9%, and 32.7%, respectively, at baseline and 31.0%, 30.8%, and 31.3%, respectively, at month 3. The %HEX in the study eye of patients in the netarsudil QD, netarsudil BID, and timolol groups was 59.6%, 59.4%, and 59.0%, respectively, at baseline and 59.4%, 58.7%, and 59.7%, respectively, at month 3. Endothelial cell density, CV, and %HEX values were similar in the netarsudil QD and BID groups relative to timolol at both baseline and month 3. There were no statistically significant between-group differences in changes from baseline to month 3 in ECD, CV, or %HEX between either of the netarsudil groups and the timolol group. Within groups, CV declined in a statistically significant fashion from baseline to month 3 by 1.4% to 2.1% (P < 0.001), and %HEX increased by a statistically significant amount (0.7%, P = 0.030) in the timolol group. These small changes in %HEX (from a mean of 59.03% to 59.73%) were unlikely to be of clinical significance. No statistically significant changes in ECD were seen in any group.

Corneal edema, a possible clinical consequence of reduction in ECD, was reported in only 1 of the 393 eyes in this analysis (0.25%), and in that eye, ECD was 2774 cells/mm² at baseline and 2815 cells/mm² at month 3. This eye, in the netarsudil BID group, experienced mild corneal edema that was assessed as unlikely to be related to study medication and that resolved with sequelae without discontinuation of netarsudil.

### Discussion

Changes in ECD and cellular morphology observed in eyes receiving netarsudil 0.02% dosed QD or BID were statistically similar to those observed in eyes receiving timolol 0.5% dosed BID. The magnitude of ECD changes from baseline to month 3 was not clinically significant and likely represents the known variability of interpretation of corneal endothelial specular microscopy images, which is on the order of 10% for ECD.21 No clinical adverse events were observed that were likely related to changes in ECD in any eye in this analysis.

The OHTS data revealed no effect of topical IOP-lowering medications on ECD.21 An analysis of approximately 20,000 eye bank eyes also found no differences in ECD of corneas from donors who did and did not use topical glaucoma medications; further analysis found no effect of any specific drug class (prostaglandin, beta-blocker, carbonic anhydrase inhibitor, alpha agonist, or miotic) and no effect of single versus multiple medication use.23-25 Of note, there were no ROCK inhibitors approved for use in the United States during the time that these studies were conducted. The potential for ROCK inhibitors to affect corneal health is suggested by results of preclinical, animal, and human studies demonstrating that this class of drugs promotes corneal endothelial cell proliferation and migration, protects against apoptosis, and enhances endothelial cell wound healing.26-35 The clinical significance of these
preliminary findings related to topical netarsudil therapy for glaucoma is unknown. In the recent MERCURY 2 clinical trial comparing the fixed combination of netarsudil and latanoprost with each of its constituents, no changes in ECD, CV, or %HEX were seen in any of the 3 groups after 3 months of therapy.35 The current study further demonstrates the lack of ECD changes with short-term netarsudil therapy.

ROCKET-2 is the largest and longest double-masked, randomized, active-controlled clinical study to evaluate the effects of topical ROCK inhibitor therapy on the human corneal endothelium. Given the enormous global burden of glaucoma—estimated to be 64 million cases and projected to grow to 112 million by 204037—full characterization of the safety effects of glaucoma therapy is of value. An additional strength of this study is the use of an independent reading center where corneal endothelium images were interpreted in masked fashion.

Limitations of this post hoc study analysis include its relatively short exposure duration and a potential selection bias in that not all subjects in ROCKET-2 underwent specular microscopy, although the demographics of the current sample are similar to the demographics of the full ROCKET-2 cohort.2 Additionally, because pachymetry was performed only at baseline (for eligibility purposes), we cannot present data on changes in central corneal thickness over time.

In conclusion, changes in ECD and corneal endothelial cell morphology, CV, and %HEX were negligible after 3 months of topical therapy with netarsudil 0.02% dosed QD or BID and were statistically similar to the changes seen with topical timolol 0.5% BID in this clinical trial.

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References


Footnotes and Financial Disclosures

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H.S.: Employees – Aerie Pharmaceuticals.
The sponsor, Aerie Pharmaceuticals, Inc, participated in the design and conduct of the study; data collection, management, and interpretation; and preparation, review, and approval of the manuscript.
The study was registered at clinicaltrials.gov before the enrollment of any subjects (www.clinicaltrials.gov; identifier: NCT02207621).

HUMAN SUBJECTS: Human subjects were included in this study. The study protocol was reviewed and approved by all relevant ethics boards. All participants provided informed consent in writing. The study was conducted in full accordance with the Declaration of Helsinki and the Health Information Portability and Accountability Act.
No animal subjects were used in this study.

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Conception and design: Mundorf, Mah, Sheng, Heah
Data collection: Mundorf, Mah, Sheng, Heah
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Obtained funding: Mundorf, Mah, Sheng, Heah
Overall responsibility: Mundorf, Mah, Sheng, Heah

Abbreviations and Acronyms:
BID = twice daily; CV = coefficient of variation; ECD = endothelial cell density; %HEX = percent of hexagonal cells; IOP = intraocular pressure;
OAG = open-angle glaucoma; OHTN = ocular hypertension; OHTS = Ocular Hypertension Treatment Study; QD = once daily; ROCK = Rho kinase; ROCKET = Rho Kinase Elevated IOP Treatment Trial.

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