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Subclinical Increased Anterior Stromal Reflectivity With Topical Taprenepag Isopropyl

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Abstract

Purpose—To assess the effect of topical taprenepag isopropyl on each layer of the cornea by confocal microscopy.

Methods—Thirty-two ocular hypertensive or glaucoma patients were randomized into a 2-period, crossover study of 14 days of 0.1% taprenepag alone and in unfixed combination with 0.005% latanoprost (combination therapy). Baseline and sequential slit-lamp biomicroscopy, fluorescein staining, central ultrasonic pachymetry, and confocal microscopy were performed. Confocal images were analyzed for the density of the central superficial and basal epithelium, midstromal keratocytes, and endothelium, as well as endothelial coefficient of variation and percentage of hexagonal cells, and reflectivity of anterior stromal and midstromal layers.

Results—Corneal staining increased from baseline, reaching a peak at day 13 (69% and 63% of subjects treated with monotherapy and combination therapy, respectively), which resolved by day 35. A statistically significant increase in mean corneal thickness for both eyes and both treatments occurred on days 7 and 13 (range, 20–27 μm ; $P < 0.001$) but recovered ($-6 \mu\text{m}$) by day 35. No statistically significant changes were observed in the basal epithelial, midstromal, or endothelial cells. Mean ratio of average reflectivity of anterior stroma to midstroma increased on days 13 and 35 in period 1 for each treatment (range, 1.2–1.9; $P < 0.001$), and this increase persisted during period 2.

Conclusions—Anterior stromal reflectivity may remain increased even when biomicroscopic and confocal images of corneal layers remain normal or have recovered after topical taprenepag. This subclinical measure may be useful to detect a persistent adverse effect of a topical agent on the cornea.

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Keywords

confocal microscopy; cornea; taprenepag isopropyl

Taprenepag isopropyl (PF-04217329) is a highly selective EP₂ receptor agonist that reduces intraocular pressure (IOP) in animals and humans.¹⁻³ In a 2-stage, phase 2 clinical trial of taprenepag, 28 days of monotherapy reduced IOP by approximately 6.5 mm Hg, comparable with 0.005% latanoprost in ocular hypertensive and open-angle glaucoma subjects.¹ However, this positive efficacy was accompanied by treatment-related adverse events of conjunctival hyperemia, photophobia, and an increase in corneal thickness of mild to moderate severity within 7 days of dosing. The increased corneal thickness did not seem to be related to the dose of taprenepag or whether it was monotherapy or combination therapy. The cornea returned to its baseline thickness within approximately 1 month of discontinuing the medication.¹

The formulation of taprenepag in these studies contained benzalkonium chloride, which has been shown to increase corneal thickness⁴; however, taprenepag vehicle and 0.005% latanoprost controls contained equivalent concentrations of benzalkonium chloride and did not induce significant changes in corneal thickness.¹ In addition, these formulations of taprenepag did not induce increased corneal thickness in rabbits or dogs.² Only at doses approximately 10 times the taprenepag dose tested clinically was an increase in corneal thickness observed in monkeys.³ This increase in corneal thickness in monkeys resolved within 2 weeks after discontinuation of taprenepag. Neither in vivo specular microscopy nor in vitro histological or transmission electron microscopic examination of the corneas of the monkeys that were killed revealed any taprenepag toxicity to the cornea or its endothelium.³

Confocal microscopy has emerged as an invaluable tool in the evaluation and diagnosis of many ocular surface disorders because it allows for a noninvasive, high-magnification evaluation of the corneal layers. Due to its ability to produce images in vivo at a microstructural level, it is increasingly used in both laboratory and clinical settings. Multiple in vivo animal and human studies have demonstrated that confocal microscopy is a reliable and effective method for detecting microscopic corneal abnormalities of the ocular surface.⁵⁻⁸ However, this technology has had limited use for the assessment of a topical medication on other layers of the cornea. The purpose of this study was to determine the etiology of taprenepag-related increase in corneal thickness with standard clinical testing along with analysis of in vivo confocal microscopic scan of the entire cornea. In particular, the measurement of changes in stromal reflectivity, as an indirect indicator of changes in corneal hydration, was explored to detect possibly subtle changes in this parameter that could provide an insight into the change in corneal thickness not clinically notable.

The plan was to assess quantitatively and qualitatively confocal images of the corneal epithelium, midstromal keratocytes, and endothelium. In addition, the average light intensity reflected from the anterior stroma and midstroma was compared to determine whether the increased corneal thickness was due to a disturbance of the corneal epithelium or endothelium affecting this light intensity. If the epithelium was the origin, the anterior stroma should be excessively hydrated and scatter more light than the midstroma. If the

endothelium was the origin, then the reverse should be true. By measuring the average light intensity, reflectivity, of the anterior stromal and midstromal images, the etiology of the increase in corneal thickness, and therefore hydration, should be suggested. Assessment of stromal reflectivity is more sensitive to subtle changes in corneal hydration than reflectivity of the superficial and/or basal corneal epithelium because of the normal increased light scattering from these layers and the potential confounding effect of cellular morphological change.

MATERIALS AND METHODS

Subjects

A randomized, double-masked (subject and investigator), sponsor-open, single-center 2-period crossover study in 30 subjects with ocular hypertension and open-angle glaucoma was conducted to evaluate the 24-hour IOP, ocular perfusion pressure, and corneal effects of 0.1% taprenepag as monotherapy and in an unfixed combination with 0.005% latanoprost. The protocol and informed consent were approved by the investigational review board at the investigational site, and the study was conducted in compliance with the Declaration of Helsinki and in compliance with all International Conference on Harmonization Good Clinical Practice Guidelines.

After obtaining informed consent, the subjects (mean age, 67 years; range, 45–85 years) had a screening ophthalmic examination and then began a washout period of 3 to 28 days of all previous ocular antihypertensive medications. The washout period was 3 days (± 1 day) if the subject was not taking ocular antihypertensive medications; 5 days (± 1 day) for miotics and carbonic anhydrase inhibitors (eg, Azopt, Trusopt, and Pilocarpine); 14 days (± 1 day) for alpha and alpha/beta agonists (eg, Iopidine, Alphagan, and Propine); and 28 days (± 1 day) for prostaglandins, beta-antagonists, and combination drugs (eg, Xalatan, Timolol, and Cosopt).

After the washout period, the subjects were required to have a baseline IOP ≥ 22 mm Hg at 8 AM and central corneal thickness ≥ 600 μ m for inclusion in the study. Subjects were excluded if they had a history of dry eyes, corneal edema, uveitis or biomicroscopic evidence of punctate keratitis, corneal inflammation, or evidence of endothelial disease (eg, Fuchs dystrophy, guttae, pigment on the endothelium, Krukenberg spindle).

Procedures and Measurements

Eight days before commencing treatment in each study period, all subjects were admitted to the clinical research unit (West Coast Clinical Trials, Cypress, CA) and confined for 24 hours to assess baseline IOP and blood pressure every 2 hours (except at 2 AM). After the 24-hour assessment in period 1, subjects were randomly assigned to 1 of the 2 treatment sequences. In the first sequence, subjects received topical ocular taprenepag 0.01% plus 0.005% latanoprost (hereafter referred to as combination therapy) in the first period and taprenepag (0.01%) plus latanoprost vehicle (hereafter referred to as monotherapy) in the second period. In the second sequence, subjects received monotherapy in the first period and

combination therapy in the second period. A schematic of the study design is shown in Figure 1.

Subjects started treatment 7 days after the 24-hour baseline assessment (day 1) to permit corneal recovery from repeated tonometry. On day 1, after an ophthalmic examination, the subjects of both treatment sequences began once-daily dosing of study drugs at 8 AM for 14 days. Subjects self-administered 1 drop of latanoprost or vehicle to each eye followed by 1 drop of taprenepag 5 minutes later.

Complete ophthalmic examinations that included best-corrected visual acuity, manifest refraction, slit-lamp biomicroscopy, ophthalmoscopy, and applanation tonometry were performed: at screening and during both periods on days 1, 7, 13, 16, 21, 28, and 35. In addition, the following serial tests were performed.

Corneal Staining

Corneal staining was performed at screening and on days 1, 7, 13, 16, 21, 28, and 35 immediately after slit-lamp biomicroscopy and before confocal microscopy, IOP determination, and pachymetry. Using a micropipette, 5 μ L of 2.0% nonpreserved sodium fluorescein was applied to the lower conjunctival sac of each eye. Immediately after instilling fluorescein, the subject was instructed to blink naturally, without squeezing, several times to distribute the fluorescein. Two minutes after fluorescein instillation, areas of corneal staining were recorded according to the standardized National Eye Institute scoring system of 0 to 3 for each of 5 areas of the cornea.⁹

Pachymetry

Pachymetry was performed on all subjects using an ultrasonic pachymeter (Pachmate 55; DGH Technologies, Inc, Exton, PA) on the same visits during which corneal staining was assessed. An average of 25 measurements of the central cornea was performed, with the subjects in the sitting position and fixating on a target straight ahead.

Confocal Microscopy

Corneal confocal microscopy was performed on days 1, 13, and 35 before IOP determination and pachymetry. After placement of a drop of the topical anesthetic proparacaine, a drop of Genteal gel (Novartis) was placed on the central cornea. A z-ring was not used to avoid disturbing the corneal epithelium. The objective of the confocal microscope (ConfoScan 4; Nidek Technologies, Srl, Padova, Italy) was placed in contact with the gel. Three separate scans of the entire central cornea from the endothelium to the epithelium in 5- μ m steps were obtained from each eye. The images were electronically stored and sent to the Case Cornea Image Analysis Reading Center (Cleveland, OH) for masked evaluation.

Independent Evaluation of Corneal Confocal Images

Cell Density by Layer and Endothelial Morphometry—The Cornea Image Analysis Reading Center imported the confocal images into Konan Analysis software (Konan KSS300 S v2.62; Konan Medical, Inc). The Konan Center method was used to analyze

endothelial cell density (ECD) and the endothelial morphology.¹⁰⁻¹² Two independent readers selected the largest area of contiguous cells in the image where the centers could be accurately distinguished and marked the center of all contiguous, analyzable cells. Mean cell area, ECD (cell density per square millimeter), coefficient of variation (CV), and percentage of hexagonal cells (%HEX) were determined from 3 images of each eye of the subjects, and the means and SD were determined. If the values observed by the 2 readers differed by >5% for ECD, >15% for CV, and/or >15% for %HEX, independent adjudication was performed by a third reader.

Using the same Konan Center Method, a single reader assessed the mean cell density and standard deviation of the basal and superficial epithelium and midstromal keratocytes of 3 separate images from the scans of each eye of the subjects. Finally, qualitative changes from baseline to the posttreatment time points were graded none, mild, moderate, or severe changes in cell size, shape, appearance of nuclei, cell membranes, or cell membrane contacts. Specifically, for the endothelium, the cells were assessed for mild to moderate changes in polymegathism, pleomorphism, reflective material, and/or guttae. Change in the midstromal keratocytes was assessed as an increase in oval or spherical cells, including mild (a <20% increase), moderate (a >20%–60% increase), and severe (a >60% increase). Basal epithelial health was assessed for mild to moderate changes in the appearance of cell membranes or cell contacts. Finally, change in superficial epithelial quality was assessed for a mild, moderate, or severe increase in either cell size or reflectivity.

Reflectivity

The images of each of the confocal scans were numbered from Descemet membrane to the superficial epithelium. The reflectivity of each layer was assessed as follows:

1. Anterior stroma: The image number that defined the boundary between the corneal stroma and Bowman membrane, just posterior to the corneal epithelium, was identified. Based on this image number, the mean image intensity of 10 consecutive images posterior to Bowman membrane, approximately 50 μm of the proximal anterior corneal stroma, was averaged.
2. Midstroma: The mid image number between Descemet and Bowman membranes was assumed to be the middle of the corneal stroma. The mean intensity of that image and the mean intensity of 12 images on either side of this midpoint (a total of 25 images, 125 μm of midstroma) were averaged.

With serial measurements of reflectivity, a change in anterior stromal and midstromal reflectivity could be due to differences in the light intensity setting or alignment of the confocal microscope between examinations. Specifically, if the light intensity setting was increased and/or the alignment of the confocal microscope was altered, the reflectivity of both the anterior stroma and midstroma would increase independent of any pathophysiological change. To address this possible artifact, the ratios of the reflectivity of the anterior stroma and midstroma were compared.

Statistical Analysis

All data analyses were carried out for the intent-to-treat (ITT) population, which consisted of all randomized subjects who received at least 1 dose of study medication. The study eye was defined as the eye with higher average IOP measurement at 8^{AM} (across eligibility visit and day 8 visit). In the event that 8^{AM} measurements were equal, the right eye was then designated as the study eye.

For all parameters, change from baseline was calculated for each eye (study eye and fellow eye) for each subject by study day and treatment period. One-sample *t* test was applied to evaluate statistical significance of the mean changes using statistical software SAS 9.2 (SAS Institute, Cary, NC). *P* values were not adjusted for multiplicity due to the exploratory nature of the study.

RESULTS

Mean corneal thickness for both eyes was similar between treatments at baseline, with a mean range of 561 to 563 μm . Increases in corneal thickness from baseline across treatments were most pronounced at days 7, 13, and 16 posttreatment (mean ranged from 20 to 27 μm). The mean increase in corneal thickness was similar between combination therapy and monotherapy, with a maximum mean increase of 26 μm for monotherapy and 27 μm for combination therapy on day 7. For both study treatments, mean corneal thickness decreased after the study treatment was stopped. At day 35, mean corneal thickness was 5 to 6 μm greater than baseline for monotherapy and 2 to 5 μm greater than baseline for combination therapy (Fig. 2A).

Mean corneal staining score of both eyes for monotherapy and combination therapy increased to a peak of 1.0 at day 13 and returned to baseline by day 35 (Fig. 2B). For those subjects with an increase in total corneal staining scores of ≥ 3 units, it was most pronounced at days 7, 13, and 16 (percent of subjects ranged from 10.0 to 17.2). The corneal staining scores began to decrease by day 21 and had returned to baseline by day 35.

Mean baseline ECD, CV, and %HEX for both eyes of the subjects was 2530 cells per square millimeter, 30.2, and 58.3, respectively. There were no statistically significant or clinically meaningful changes in these parameters or in the qualitative and quantitative assessments of the midstromal keratocytes and the basal epithelial cells over the entire study period. A limited number of superficial epithelial images of sufficient quality showed cell enlargement and irregularity with both treatments. Representative images of the abnormal, superficial corneal epithelium and normal basal epithelium, midstroma, and endothelium from a subject with an increase in corneal staining score and corneal thickness during treatment are presented (Fig. 3).

The light intensity of the confocal microscope was recorded for each image captured and did not vary more than 15% (range, 87%–99%) between scans. At baseline, the average anterior stromal reflectivity was greater than that of the average midstromal reflectivity. The mean baseline intensity level of anterior stromal reflectivity was 19.6 and 19.5 (combination therapy and monotherapy, respectively). The mean baseline intensity level of midstromal

reflectivity in the study eye was 12.8 and 14.0 (combination therapy and monotherapy, respectively).

Anterior stromal reflectivity and midstromal reflectivity increased after both combination therapy and monotherapy. The mean change of anterior stromal reflectivity from baseline to day 35 for both eyes was 63.0 and 74.8 (combination therapy and monotherapy, respectively). The mean change of midstromal reflectivity from baseline to day 35 for both eyes was 66.3 and 64.8 (combination therapy and monotherapy, respectively). Finally, the ratio of anterior stromal reflectivity to midstromal reflectivity increased in period 1. The increase in reflectivity ratio in period 1 seemed to be unrelated to changes in light intensity of the confocal microscope. During period 2, no further increase in this ratio was observed (Fig. 4).

DISCUSSION

In vivo confocal microscopy has emerged as a useful tool in both the laboratory and clinical setting for the evaluation of drug-induced ocular surface toxicity, producing a noninvasive, high-magnification image of the cornea and conjunctiva at the microstructural level that can be repeatedly obtained for sequential studies.¹³⁻¹⁶ This technology has many advantages over other in vivo methods,¹⁷⁻²⁴ in that along with its noninvasive approach, serial studies can be performed on all layers of the cornea and/or conjunctiva and reproducible, masked measurements can be obtained with the help of a reading center. Confocal microscopy has been reported to be advantageous for use in the assessment of ocular surface changes in Sjogren syndrome,²⁵ dry eye disease,²⁶ and superior limbic keratoconjunctivitis.²⁷ In the current study, we used in vivo confocal microscopy of the entire central cornea as the primary method for the qualitative and quantitative assessment of drug-induced corneal toxicity associated with an investigational drug, taprenepag isopropyl.

In our study, there was no confocal microscopic evidence of drug-induced toxicity to the corneal endothelium, midstromal keratocytes, or basal epithelial cells from either taprenepag monotherapy or taprenepag in combination with latanoprost. Thus, the increase in corneal thickness we again observed with taprenepag¹ does not seem to be due to endothelial dysfunction. In contrast, the subtle increase in corneal staining and ratio of average anterior stromal reflectivity to average midstromal reflectivity suggests that taprenepag may be disturbing the superficial corneal epithelium. This is consistent with the cell enlargement and irregularity noted in a few images of sufficient quality to assess the superficial corneal epithelium (Fig. 3). We were, however, unable to obtain high-quality images of the superficial corneal epithelium consistently from all subjects and therefore could not reliably assess changes in superficial corneal epithelial cell size or density. Further research is required to devise a method for reliably imaging the superficial corneal epithelium.

The determination of stromal light intensity proved to be an unexpected but useful measure compared with morphologic changes in the various cellular layers to account possibly for the change in thickness observed. However, reflectivity of the superficial and/or basal corneal epithelium was not useful because of the normal increased light scattering from these layers and the potential confounding effect of cellular morphological change. By

comparing the average light intensity reflected from the anterior stroma and midstroma, we believe that we were able to sort out whether the thickness change was due to epithelial or endothelial dysfunction. If the epithelium was the origin, the anterior stroma should be excessively hydrated and scatter more light than the midstroma. If the endothelium was the origin, then the reverse should be true. In fact, the increase in the ratio of anterior stromal light intensity to midstromal light intensity suggested that the origin of the hydration disturbance and increase in thickness was due to an epithelial disturbance affecting the anterior stroma.

The increase in corneal staining peaked at day 13 of the 14-day treatment, whereas the increase in ratio of anterior stromal reflectivity to midstromal reflectivity did not peak until day 35, 21 days after treatment discontinuation. A possible explanation for this difference is the increase in corneal hydration on day 13 is not sufficient to be detected by the confocal microscope. As the superficial epithelium begins to heal after the treatment is stopped and the cornea deturgescens, the stromal collagen fibers may become more irregular, increasing light scattering and consequently stromal reflectivity²⁸ at day 35. None of the subjects had a change in best-corrected visual acuity or had any visual symptoms at day 35. This suggests that these reflectivity changes are not clinically meaningful; however, whether these subtle stromal changes persist or resolve with time may require a longer follow-up.

It is possible that the increase in reflectivity ratio was due to variations in movement of the eye in the z direction relative to the confocal microscope. The depth of field of the confocal microscope is approximately 25 μm ,²⁹ making it conceivable that compared layers were not in exactly the same plane. However, the consistent mean increase in the reflectivity ratio from day 35 of period 1 to the end of period 2 in the taprenepag monotherapy-treated study and fellow eyes (Fig. 4A) makes it unlikely that the observed increase in reflectivity ratio was an artifact. The addition of eye tracking to the confocal microscope would significantly enhance the precision and reliability of the instrument.

In summary, confocal microscopic scanning of the entire cornea can be a useful tool to dissect the etiology of subclinical corneal changes. Sequential qualitative, quantitative, and reflectivity analyses of each corneal layer provide a sensitive tool for detecting potential topical corneal drug toxicity, which may improve drug safety evaluation. Further comparative studies are required to validate the technique.

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REFERENCES

1. Schachar RA, Raber S, Courtney R, et al. A phase 2, randomized, dose-response trial of taprenepag isopropyl (PF-04217329) versus latanoprost 0.005% in open-angle glaucoma and ocular hypertension. *Curr Eye Res.* 2011; 36:809–817. [PubMed: 21851167]

2. Prasanna G, Carreiro S, Anderson S, et al. Effect of PF-04217329 a pro- drug of a selective prostaglandin EP(2) agonist on intraocular pressure in preclinical models of glaucoma. *Exp Eye Res.* 2011; 93:256–264. [PubMed: 21376717]
3. Yanochko, GM.; Khoh-Reiter, S.; Lee, D., et al. Nonclinical effects of taprenepag isopropyl (PF-04217329), a selective EP2 agonist, and its active metabolite on the cornea; Presented at: World Glaucoma Congress; Paris, France. June 29-July 2, 2011; Abstract 759
4. Kennah HE II, Hignet S, Laux PE, et al. An objective procedure for quantitating eye irritation based upon changes of corneal thickness. *Fundam Appl Toxicol.* 1989; 12:258–268. [PubMed: 2714526]
5. Ly LT, Cavanagh HD, Petroll WM. Confocal assessment of the effects of fourth-generation fluoroquinolones on the cornea. *Eye Contact Lens.* 2006; 32:161–165. [PubMed: 16845259]
6. Price MO, Price FW Jr, Maclellan D. Effect of gatifloxacin 0.3% and moxifloxacin 0.5% ophthalmic solutions on human corneal epithelium following 2 dosing regimens. *J Cataract Refract Surg.* 2005; 31:2137–2141. [PubMed: 16412928]
7. Labbé A, Pauly A, Liang H, et al. Comparison of toxicological profiles of benzalkonium chloride and polyquaternium-1: an experimental study. *J Ocul Pharmacol Ther.* 2006; 22:267–278. [PubMed: 16910868]
8. Alomar TS, Nubile M, Lowe J, et al. Corneal intraepithelial neoplasia: in vivo confocal microscopic study with histopathologic correlation. *Am J Ophthalmol.* 2011; 151:238–247. [PubMed: 21168809]
9. Lemp MA. Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes. *CLAO J.* 1995; 21:221–232. [PubMed: 8565190]
10. Azar RG, Holdbrook MJ, Lemp M, et al. Two-year corneal endothelial cell assessment following INTACS implantation. *J Refract Surg.* 2001; 17:542–548. [PubMed: 11583224]
11. Edelhauser HF, Sanders DR, Azar R, et al. Corneal endothelial assessment after ICL implantation. *J Cataract Refract Surg.* 2004; 30:576–583. [PubMed: 15050252]
12. Sheng H, Parker EJ, Bullimore MA. An evaluation of the ConfoScan3 for corneal endothelial morphology analysis. *Optom Vis Sci.* 2007; 84:888–895. [PubMed: 17873775]
13. Martone G, Frezzotti P, Tosi GM, et al. An in vivo confocal microscopy analysis of effects of topical antiglaucoma therapy with preservative on corneal innervation and morphology. *Am J Ophthalmol.* 2009; 147:725–735. [PubMed: 19181302]
14. Cavanagh HD, El-Agha MS, Petroll WM, et al. Specular microscopy, confocal microscopy, and ultrasound biomicroscopy: diagnostic tools of the past quarter century. *Cornea.* 2000; 19:712–722. [PubMed: 11009323]
15. Jester JV, Maurer JK, Petroll WM, et al. Application of in vivo confocal microscopy to the understanding of surfactant-induced ocular irritation. *Toxicol Pathol.* 1996; 24:412–428. [PubMed: 8864183]
16. Mathers WD, Petroll WM, Jester JV, et al. Basic science and applications of in vivo microscopy. *Curr Opin Ophthalmol.* 1995; 6:86–94. [PubMed: 10150888]
17. Wilhelmus KR. The Draize eye test. *Surv Ophthalmol.* 2001; 45:493–515. [PubMed: 11425356]
18. Gupta KC, Chambers WA, Green S, et al. An eye irritation test protocol and an evaluation and classification system. *Food Chem Toxicol.* 1993; 31:117–121. [PubMed: 8449456]
19. Secchi A, Deligianni V. Ocular toxicology: the Draize eye test. *Curr Opin Allergy Clin Immunol.* 2006; 6:367–372. [PubMed: 16954791]
20. U.S. National Academy of Sciences. Principles and Procedures for Evaluating the Toxicity of Household Substances. National Academy of Sciences; Washington, DC: 1977.
21. Hayes, AW. Principles and Methods of Toxicology. 5th ed.. Informa Healthcare USA; New York, NY: 2011.
22. Savitsky DZ, Fan VC, Yildiz EH, et al. Fluorophotometry to evaluate the corneal epithelium in eyes undergoing contact lens corneal reshaping to correct myopia. *J Refract Surg.* 2009; 25:366–370. [PubMed: 19431927]
23. Lass JH, Pavan-Langston D, Park NH. Aciclovir and corneal wound healing. *Am J Ophthalmol.* 1979; 88:102–108. [PubMed: 463999]

24. Burstein NL. Corneal cytotoxicity of topically applied drugs, vehicles and preservatives. *Surv Ophthalmol*. 1980; 25:15–30. [PubMed: 6998034]
25. Wakamatsu TH, Sato EA, Matsumoto Y, et al. Conjunctival in vivo confocal scanning laser microscopy in patients with Sjögren syndrome. *Invest Ophthalmol Vis Sci*. 2010; 51:144–150. [PubMed: 19696170]
26. Zhang X, Chen Q, Chen W, et al. Tear dynamics and corneal confocal microscopy of subjects with mild self-reported office dry eye. *Ophthalmology*. 2011; 118:902–907. [PubMed: 21146227]
27. Kojima T, Matsumoto Y, Ibrahim OM, et al. In vivo evaluation of superior limbic keratoconjunctivitis using laser scanning confocal microscopy and conjunctival impression cytology. *Invest Ophthalmol Vis Sci*. 2010; 51:3986–3992. [PubMed: 20375341]
28. Jester JV, Ghee Lee Y, Li J, et al. Measurement of corneal sublayer thickness and transparency in transgenic mice with altered corneal clarity using in vivo confocal microscopy. *Vision Res*. 2001; 41:1283–1290. [PubMed: 11322973]
29. McLaren JW, Bourne WM, Patel SV. Automated assessment of keratocyte density in stromal images from the ConfoScan 4 confocal microscope. *Invest Ophthalmol Vis Sci*. 2010; 51:1918–1926. [PubMed: 19892869]

FIGURE 1.

Schematic of the crossover study. Subjects were evaluated on days 16, 21, 28, and 35 after 14 days of treatment in each period.

FIGURE 2.

Change from baseline in corneal thickness (A) and corneal staining (B) associated with monotherapy (0.01% taprenepag + latanoprost vehicle) and combination therapy (0.01% taprenepag + 0.005% latanoprost). The error bars are standard deviations.

FIGURE 3.

Representative confocal images of a patient who had an increase in corneal staining score and corneal thickness during treatment. At baseline, period 1, day 1: (A) superficial epithelium with normal hyper-reflective small nuclei, (C) normal basal epithelium, (E) normal mid-stroma with keratocytes, and (G) normal endothelium. At the end of the study, period 2, day 35: (B) superficial epithelium with abnormally enlarged nuclei that have markedly reduced reflectivity. In addition, the superficial epithelial cells are enlarged and

irregular. The other layers of the cornea (D) basal epithelium, (F) mid-stroma and keratocytes, and (H) endothelium seem to be unaffected by the taprenepag treatment.

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Box Plots of Reflectivity Ratio (Anterior/Mid Stroma) Overtime: PF329 + latanoprost vehicle

A

Box Plots of Reflectivity Ratio (Anterior/Mid Stroma) Overtime: PF329 + latanoprost 0.005%

B

FIGURE 4.

Box plots of ratio of average anterior stromal reflectivity to average midstromal reflectivity for (A) taprenepag monotherapy and (B) combination therapy.