Effects of Topical Phenylephrine and Tafluprost on Optic Nerve Head Circulation in Monkeys with Unilateral Experimental Glaucoma

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PURPOSE. To compare the effects of a topical alpha agonist (vasoconstrictor) and a prostaglandin receptor (FP) agonist (vasodilator) on circulation in the optic nerve head (ONH) in experimental glaucomatous and normal eyes of monkeys.

METHODS. Tissue blood velocity in the ONH (NB ONH) was determined using the laser speckle method in both eyes of eight normal cynomolgus monkeys under systemic anesthesia for 180 minutes after bilateral instillation of 5% phenylephrine. The effect of 0.0015% tafluprost, a potent FP agonist, was also studied after single and once-daily 7-day instillations. Measurements were repeated in both eyes of the eight monkeys after establishment of unilateral laser-induced glaucoma.

RESULTS. NB ONH decreased significantly in both eyes of normal monkeys 30 to 120 minutes after phenylephrine instillation by a maximum of 9% to 11% (P < 0.05) without significant change in intraocular pressure (IOP). A similar decrease in NB ONH was found in non–laser-treated eyes in glaucomatous monkeys despite the absence of significant changes in contralateral experimental glaucomatous eyes. NB ONH increased by 16% (P < 0.05) 60 minutes after a single instillation and also after 7-day repeated instillations of tafluprost in both eyes of normal monkeys. A similar increase in NB ONH occurred in both eyes after the establishment of unilateral glaucoma but was completely abolished by 5 mg/kg indomethacin injected intravenously 15 minutes after tafluprost instillation. Tafluprost significantly reduced IOP only in experimental glaucomatous eyes by 34%.

CONCLUSIONS. The ONH vasculature in glaucomatous and normal eyes reacts differently to an exogenous alpha agonist, whereas it reacts similarly to an FP agonist. (Invest Ophthalmol Vis Sci. 2010;51:4117–4124) DOI:10.1167/iovs.10-5218

Optic circulation is impaired in patients with open-angle glaucoma (OAG) or ocular hypertension,1–6 and compromised local circulation is probably correlated with visual field deterioration.7–9 Thus, improvement of local ocular circulation may be beneficial in the treatment of glaucomatous optic neuropathy.

There are ethical difficulties in human studies on ocular circulation including pharmacologic challenges, especially in OAG patients, in whom such trials can harm their already damaged optic nerves. Further, differences in results between healthy control subjects and OAG patients must be interpreted cautiously because of apparent interindividual differences in factors affecting systemic or local circulation (e.g., age-related vascular changes, blood pressure, blood levels of various vasoactive substances, and reactivity to them). Studies in patients with unilateral glaucoma would circumvent these problems, but ethical and practical difficulties in recruiting such patients for blood flow experiments remain. Laser-induced experimental glaucoma may not provide identical pathologic changes in human glaucoma, but experiments in primates with unilateral glaucoma would allow researchers to avoid ethical problems, difficulty in recruiting patients with unilateral glaucoma for pharmacologic experiments, and differences between healthy control subjects and glaucoma patients in many systemic factors influencing circulation.

Several topically applied drugs potentially improve optic nerve head (ONH) circulation.10–15 Differences in reactivity between normal and glaucomatous eyes to pharmacologic challenges would be of clinical interest and might improve our understanding of the pathophysiologic of glaucomatous ONH damage. We previously found that the topical prostaglandin (FP) receptor agonists latanoprost and travoprost improve ONH circulation in humans and in experimental animals, probably by penetrating locally and stimulating local endogenous vasodilating prostaglandins such as PG-I2,10,11,16 and the topical alpha agonist phenylephrine decreases ONH circulation, probably through local penetration and alpha adrenoreceptor stimulation.17–19

We further explore vascular pharmacopathologic aspects of glaucomatous ONH damage by comparing the effects of topical phenylephrine and tafluprost, a recently developed potent selective FP agonist,20 on ONH circulation in both eyes of cynomolgus monkeys with unilateral laser-induced glaucoma in vivo.

METHODS

Induction of Glaucoma

Experiments were performed in normal eyes of eight female cynomolgus monkeys, (weight range, 2.6–4.8 kg) and were repeated after glaucoma was induced in the left eyes; the right eyes served as controls for all investigations. Experimental procedures were conducted in accordance with the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

The left eyes of all monkeys were treated with argon blue/green laser cautery to the trabecular meshwork under systemic anesthesia, as described previously.21,22 Argon laser was applied around the trabecular meshwork for 360° (spot number, 80–110; spot size, 100-μm diameter; power, 1000 mW; exposure time, 0.2 seconds) using an argon-laser photo-coagulator (Ultima 2000SE; Coherent, Inc., Santa Bunkyo-ku, 113-0033, Tokyo, Japan; cmayama-tky@umin.ac.jp.

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Clara, CA), preventing overlap with each other. Two weeks later, the laser treatment was repeated to maintain intraocular pressure (IOP) elevation. Time ‘after laser treatment’ or ‘after IOP elevation’ should be observed from the first laser treatment.

Before and after laser treatment, IOP was measured using a calibrated pneumotonometer (Alcon, Fort Worth, TX), and anterior segments of the eye were examined with a slit lamp microscope every week under topical anesthesia alone to avoid side effects of repeated systemic anesthesia. When monkeys seemed reluctant, weak sedation was induced by intramuscular administration of 5 mg/kg ketamine.

Morphology of ONH was evaluated by confocal scanning laser tomography (Heidelberg Retina Tomograph [HRT], version 2.01; Heidelberg Engineering, Heidelberg, Germany), using a rigid contact lens in both eyes. HRT examination and fundus imaging, using a fundus camera (TRC-W; Topcon Corporation, Tokyo, Japan), were conducted simultaneously before laser treatment and at the time of phenylephrine or tafluprost instillation after the induction of glaucoma. HRT measurements were carried out as previously reported, and SD of the integrated images <50 μm was adopted. Disc contour line was determined by an experienced operator (TS) in all images with reference to the fundus photographs taken simultaneously. The standard reference plane was 50 μm posterior to the mean height of the disc contour located temporally between 350° and 356°.

Method of ONH Circulation Measurement

Effects of the topical drugs on ONH circulation were studied using the normalized blur (NB) value, a quantitative index of tissue blood velocity obtained by the noninvasive laser speckle method. It allows noncontact and noninvasive two-dimensional measurement of blood velocity in intraocular tissues, including ONH, using a laser beam and a fundus camera. The laser beam (wavelength 808 nm; power 2 mW) was focused on the ONH rim in the largest area free of visible vessels, and the scattered light was imaged on an image sensor where the speckle pattern of the laser appeared. NB value was measured every 0.125 seconds, and average NB in the target area (0.42 × 0.42 mm on average) was calculated as NBONH. The successive averages of NBONH for three to four heartbeats were calculated as NBONH.

The penetration depth of the infrared laser (wavelength, 811 nm) in a cat optic nerve reportedly exceeds 1 mm. NBONH is thought to represent blood circulation in the prelaminar to laminar part of the ONH, and it correlates well with the blood flow rate in the ONH determined by the hydrogen gas clearance method under various conditions with good reproducibility.

Drug Instillation and Measurement of ONH Circulation

Phenylephrine, an alpha-1 agonist, is commonly used topically for mydriasis before intraocular surgery or fundus examination. Its long-term topical administration produced significant vasoconstriction in retrolubular arterioles around ONH in rabbits, and we also found that ONH blood flow decreased in humans, monkeys, and rabbits in vivo. Its effects are thought to be mediated by the α1 receptor.

Tafluprost is a recently developed selective FP agonist with an IOP-decreasing effect reportedly equivalent to that of latanoprost. The vasodilating effect of tafluprost was demonstrated in isolated rabbit ciliary artery segments, and topical instillation of other selective FP agonists—latanoprost and travoprost—increased ONH circulation in the ipsilateral side in humans, monkeys, and rabbits.

Effects of topical phenylephrine and tafluprost on ONH blood flow were studied first in the monkeys before glaucoma induction. The following procedures were performed under systemic anesthesia with bolus intravenous 15 mg/kg pentobarbital sodium (Abbott Laboratories, North Chicago, IL) with supplemental doses of 5 mg/kg, if needed, after mydriasis was achieved with topical 0.4% tropicamide (Mydrin M; Santen Pharmaceutical, Osaka, Japan) in both eyes. IOP was measured using a calibrated pneumotonometer, and brachial arterial pressure and heart rate were continuously measured using an automatic blood pressure meter (BP-8800; Colin Medical Technology Corporation, Komaki City, Japan). Animals were allowed to rest for 30 minutes until systemic circulatory parameters stabilized.

A 20-μL drop of 5% phenylephrine hydrochloride was instilled into both eyes of each monkey at 2 PM, and NBONH, IOP, brachial arterial pressure, and pulse rate were measured before and 30, 60, 90, 120, 150, and 180 minutes after drug instillation, as reported previously. Teflon tubing was inserted into both eyes and measurement procedures were similar to those performed before glaucoma induction.

Next, glaucoma was induced in each monkey. The effects of topical phenylephrine were restudied 28 to 32 weeks after the induction of glaucoma, and the effects of topical tafluprost were restudied 4 to 8 weeks thereafter. Instillation of phenylephrine or tafluprost in both eyes and measurement procedures were similar to those performed before glaucoma induction.

Eight to 12 weeks after the experiments, NOH and IOP changes were studied 60 minutes after a single tafluprost instillation, when 5 mg/kg indomethacin was intravenously injected 15 minutes after the tafluprost instillation.

All measurements were conducted under masked conditions, and data obtained with the laser speckle method were stored in magneto-optical discs and later analyzed by another investigator masked to the drug treatment.

Statistical Analysis

NBONH change was normalized to the baseline (first measurement before drug instillation at each experiment), and normalized values were compared between the glaucomatous and control eyes or between the first and second experiments.

Paired t-test was applied to examine the difference from the baseline, and repeated-measures ANOVA and paired t-test applied to the difference between both eyes or between the eyes before and after the establishment of experimental glaucoma. When necessary, Bonferroni’s correction was used to adjust P values. P < 0.05 was considered significant.

RESULTS

Experimental Glaucoma

In laser-treated eyes, IOP rapidly became elevated in all eyes after the second laser treatment and remained significantly higher than that in control eyes. Transient corneal edema, slight hyphema, or both were noted after the second treatment in two eyes, but it disappeared in a few weeks.

Figure 1a shows the time course of mean IOP of the laser-treated and contralateral non–laser-treated eyes. Mean IOP of the laser-treated eyes measured under topical anesthesia increased up to 45 mm Hg and then decreased gradually. IOP in the laser-treated eyes was significantly higher than that in control eyes. Transient corneal edema, slight hyphema, or both were noted after the second treatment in two eyes, but it disappeared in a few weeks.

Figure 1b shows a representative fundus photograph of ONH in a glaucomatous monkey eye. Mean cup/disc area ratio and rim area values obtained with HRT were 0.077 ± 0.092 mm² and 0.18 mm² in the left eye before the laser treatment. These values were significantly altered (P < 0.05) after glaucoma induction and remained stable during the phenylephrine and tafluprost experiments, measuring 0.29 ± 0.30 mm² and 0.98 ± 0.45 mm² and 0.30 ± 0.31 mm² and 0.98 ± 0.47 mm², respectively.
Effects of Phenylephrine

Drug instillation and measurements were carried out in all monkeys. However, vital signs were not sufficiently stabilized after anesthesia in one monkey after establishment of glaucoma; those results were discarded.

In normal monkeys, NB_ONH showed a maximum decrease of 9% to 11% at 90 minutes after single phenylephrine instillation compared with the preinstillation baseline measurements in both eyes. The change was significant at 30 to 120 minutes ($P < 0.0013$–$0.023$; Bonferroni's correction), and no significant difference was present between both eyes ($P = 0.28$; Fig. 2a). IOP tended to increase slightly in both eyes, but the changes were not significant compared with the baseline at any time point ($P > 0.15$), and no significant intereye differences were noted in IOP ($P > 0.11$; Fig. 2b). Mean blood pressure was significantly increased ($P = 0.0074$; Bonferroni's correction), and pulse rate was significantly decreased ($P < 0.001$; Bonferroni's correction) at 30 minutes after phenylephrine instillation; these parameters remained stable for 180 minutes (Fig. 2b).

After the establishment of unilateral experimental glaucoma, NB_ONH did not differ significantly from baseline ($P > 0.08$; Bonferroni's correction) in glaucomatous eyes, but it showed a significant decrease at 60 to 90 minutes after phenylephrine instillation ($P = 0.0011$–$0.014$; Bonferroni's correction), with a maximum decrease of 10% in non–laser-treated control eyes, similar to that obtained in the same eye before laser treatment (Fig. 3a). The NB_ONH change in experimental glaucomatous eyes differed significantly from that in the contralateral control eyes ($P = 0.018$) or that in the same eyes before laser treatment ($P = 0.00038$), showing disappearance of the topical phenylephrine effect on ONH circulation after experimental glaucoma was established. There were no significant differences in non–laser-treated control eyes before and after laser treatment ($P = 0.32$).

IOP showed no significant change after phenylephrine instillation in both eyes, similar to the findings before laser treatment. In experimental glaucomatous eyes, IOP exceeded that of the contralateral control eyes by 2.4 to 5.6 mm Hg throughout the experimental period, with a significant difference seen 180 minutes after phenylephrine instillation ($P = 0.0064$; Bonferroni’s correction; Fig. 3b). Systemic circulatory parameters showed changes similar to those occurring before laser treatment, with an increase in blood pressure ($P = 0.051$; Bonferroni’s correction) and a decrease in pulse rate ($P = 0.011$; Bonferroni’s correction) 30 minutes after phenylephrine instillation (Fig. 3b).

Effects of Tafluprost

Drug instillation and measurements were carried out in all monkeys; results of one normal monkey were discarded because of unstable vital signs.
compared with the baseline value. (%) without significant difference between both eyes (Bonferroni’s correction). NBONH returned to the baseline level by 180 minutes after the last instillation (Fig. 4b).

After the establishment of unilateral glaucoma, NBONH increased significantly (14%; \( P = 0.0026 - 0.038; \) Bonferroni’s correction) at 60 minutes after single tafluprost instillation in control eyes, and a similarly significant increase (16%; \( P = 0.010 - 0.012; \) Bonferroni’s correction) occurred at 60 minutes in the experimental glaucomatous eyes (Fig. 5a). No significant differences were seen in NBONH changes between both eyes (\( P = 0.64 \)) or between the same eyes before and after the laser treatment (\( P = 0.16 \)). IOP in the control eyes was not significantly changed from the baseline after single tafluprost instillation (\( P > 0.45 \); Bonferroni’s correction). However, IOP in the glaucomatous eyes, which was significantly higher than that in

NBONH showed a maximum increase by 13% to 14% compared with baseline at 60 minutes after the single tafluprost instillation in both eyes before glaucoma induction. The change was significant at 60 to 120 minutes in right eyes and at 30 to 90 minutes in left eyes (\( P = 0.0007 - 0.035; \) Bonferroni’s correction). NBONH returned to the baseline level by 180 minutes after tafluprost instillation, and no significant differences were observed in NBONH changes between both eyes (\( P = 0.51 \)). After 7 days of instillation and just before the last instillation of tafluprost, NBONH was at the same level as baseline, and a significant increase was reproduced in both eyes 60 minutes after the last instillation of tafluprost (Fig. 4a).

IOP was not significantly changed in both eyes after single or 7-day repeated instillations (\( P > 0.11 \); Bonferroni’s correction) without significant difference between both eyes (\( P > 0.11 \); Bonferroni’s correction; Fig. 4b). Mean blood pressure was not significantly changed (\( P > 0.06 \); Bonferroni’s correction). Pulse rate was significantly decreased (\( P = 0.017 \); Bonferroni’s correction) at 60 minutes after tafluprost instillation and remained stable until 180 minutes Pulse rate on the seventh day was significantly lower than the baseline level (\( P = 0.016 \); Bonferroni’s correction) and remained unchanged after the last instillation (Fig. 4b).

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FIGURE 2. (a) NBONH after phenylephrine instillation in normal monkeys. Values are mean ± SE (\( n = 8 \)). *\( P < 0.05 \) with Bonferroni’s correction compared with the baseline value. (b) Mean blood pressure, pulse rate, and IOP after phenylephrine instillation in normal monkeys. Values are mean ± SE (\( n = 8 \)). *\( P < 0.05 \) with Bonferroni’s correction compared with the baseline value.

FIGURE 3. (a) NBONH after phenylephrine instillation in unilateral experimental glaucoma monkeys. Values are mean ± SE (\( n = 7 \)). *\( P < 0.05 \) with Bonferroni’s correction compared with the baseline value. (b) Mean blood pressure, pulse rate, and IOP after phenylephrine instillation in unilateral experimental glaucoma monkeys. Values are mean ± SE (\( n = 7 \)). *\( P < 0.05 \) with Bonferroni’s correction between both eyes.
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FIGURE 4. (a) NBONH after tafluprost instillation in normal monkeys. Values are mean ± SE (n = 7). *P < 0.05 with Bonferroni’s correction compared with the baseline value. (b) Mean blood pressure, pulse rate, and IOP after tafluprost instillation in normal monkeys. Values are mean ± SE (n = 7). *P < 0.05 with Bonferroni’s correction compared with the baseline value.

DISCUSSION

Experimental glaucoma models with high IOP can be created in mice, rats, and rabbits. However, only monkeys have an ONH anatomic structure and vascular system similar to those in human eyes. Although high IOP alone causes glaucomatous changes in experimental glaucoma, various lines of investigations noted similarities between experimental glaucomatous monkey eyes and human glaucomatous...
eyes. Alterations in the morphologic appearance or extracellular matrix of the ONH,\textsuperscript{44} apoptosis of retinal ganglion cells,\textsuperscript{45} retinal nerve fiber layer defects,\textsuperscript{46} neuron loss in lateral geniculate nucleus,\textsuperscript{47,48} and histochemical changes in cortical neurons\textsuperscript{49,50} have also been found in experimental glaucomatous monkey eyes.

The unilateral glaucoma model enables the contralateral non–laser-treated eyes to serve as a control, and common systemic circulatory parameters, including blood pressure, pulse rate, autonomic nervous tone, and plasma levels of various vasoactive substances between the two eyes provide an ideal platform in which to carry out a comparative study of the pharmacologic properties of ONH vascularity in experimental glaucomatous eyes.

Results of HRT examination demonstrated that significant glaucomatous change in the ONH was established and remained unchanged during pharmacologic experiments using topical phenylephrine and tafluprost. Morphologic changes in the ONH after laser treatment were relatively moderate and compatible with those reported in the previous study,\textsuperscript{21} whereas the cup/disc area ratio increased from approximately 0.08 to 0.30 in this study.

After single topical phenylephrine instillation, NB\textsubscript{ONH} was significantly decreased in both eyes of normal monkeys and in non–laser-treated control eyes of unilateral experimental glaucomatous monkeys, as previously reported.\textsuperscript{18} These results suggested that reactivity of the ONH vasculature to an alpha agonist in the non–laser-treated eyes remained unchanged after the stress associated with the experimental glaucoma induction and repeated systemic anesthesia. In contrast, for the experimental glaucomatous eyes, reactivity of the ONH vasculature to an alpha agonist was diminished unilaterally. Thus, not only quantitative, as shown by HRT measurements, but also functional alterations in the pharmacologic reaction of the vasculature in the ONH rim tissue were induced after experimental glaucoma was established.

The mechanism by which reactivity to an alpha agonist disappeared in the ONH of the experimental glaucomatous eyes is unclear. No significant differences in NB\textsubscript{ONH} were measured before or after experimental glaucoma was induced (mean NB\textsubscript{ONH} values were 9.9 and 9.8, respectively; P = 0.83, paired t-test), but, because of the morphologic changes in ONH, the NB\textsubscript{ONH} measurement location in the rim and its laser reflectivity before and after the induction of experimental glaucoma were not identical. Some phenylephrine was absorbed in the systemic circulation and might have been partially responsible for the observed effects. However, concentrations of the drug distributed topically or systemically that could act on the vasculature, nourishing the ONH rim tissue, were identical between both eyes of each animal because phenylephrine was instilled bilaterally in this study. Thus, the difference in reactivity of the ONH circulation between both eyes should be attributed to the glaucomatous changes in the ONH that was experimentally developed.

We have demonstrated that a topically instilled FP agonist diffused to the posterior ocular tissues by local penetration and significantly increased ONH circulation, presumably through endogenous production of prostaglandins in rabbits or monkeys.\textsuperscript{10,11} Tafluprost, a potent selective FP agonist, also showed a significant effect on ONH circulation in normal and control eyes of unilateral experimental glaucomatous monkeys in the present study, probably by similar local penetration. Because IOP and blood pressure did not change significantly, the effect cannot be attributed to a secondary effect of altered ocular perfusion pressure after IOP reduction. There might have been some effects of pentobarbital on ONH blood flow; however, we have confirmed that the minimum dose of additional pentobarbital injection had little effect on ocular blood flow in previous examinations in rabbits.\textsuperscript{24,51} and monkeys (unpublished data, 2007) under placebo administration using the laser speckle method. The effects of the factors corresponding to anesthesia and systemically absorbed tafluprost that might affect ocular blood flow were identical between both eyes.

Unexpectedly, the effect of tafluprost on ONH circulation remained unchanged after experimental glaucoma was established. IOP decreased significantly after tafluprost instillation in the glaucomatous eyes, and the mechanism of increase in NB\textsubscript{ONH} might have differed between normal and experimental glaucomatous eyes. However, it was probably not because of a secondary effect of decreased IOP given that the increased NB\textsubscript{ONH} returned to a pretreatment level 180 minutes after tafluprost instillation, when IOP in those eyes was still at its lowest level. Disappearance of the effect of tafluprost on the ONH circulation after indomethacin injection suggested that tafluprost probably exerts its effect through secondarily synthesized prostaglandins in vivo, as we have demonstrated in the case of latanoprost\textsuperscript{10} and travoprost.\textsuperscript{11,52}

Topical tafluprost has decreased IOP significantly in monkeys,\textsuperscript{53} however, IOP change was not significant in normal monkeys under systemic anesthesia in the present study. This discrepancy can probably be explained by the lower IOP level in normal monkey eyes in this study (mean, 12.0 mm Hg before tafluprost instillation) compared with the previous study\textsuperscript{20} (range, 21.5–22.1 mm Hg), which is compatible with the finding that significant IOP reduction occurred in the experimental glaucomatous eyes with higher IOP (mean, 25.7 mm Hg) in this study. It has been reported that IOP significantly decreased under anesthesia with pentobarbital\textsuperscript{54}; depth of anesthesia might affect IOP levels.

The fact that reaction to an FP agonist remained unchanged after experimental glaucoma was established—while response to an alpha agonist was abolished—in the local circulation in the ONH rim of the monkeys has clinical implications. It suggests that endogenous prostaglandin production and its effects on the ONH circulation were not altered in those eyes, in contrast to the apparent damage in the ONH rim tissue. The current findings in monkeys are consistent with results of prior studies in glaucoma patients, which found significantly increased blood flow after instillation of topical FP agonists in the ONH\textsuperscript{19} or retrobulbar vessels.\textsuperscript{55,57} Although the mechanism is unclear and the results in the experimental glaucomatous monkey eyes may not be directly extrapolated to human glaucomatous eyes, the fact that the ONH rim circulation of glaucoma is not sensitive to an alpha agonist vasodilation should be considered when topical or systemic drugs with α-agonistic activity are indicated in glaucoma patients.

References

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