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CLINICAL STUDY

Topical phenylephrine decreases blood velocity in the optic nerve head and increases resistive index in the retinal arteries

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Abstract

Purpose **To investigate the effects of topical phenylephrine on circulation in the optic nerve head (ONH), posterior choroids, or retina in rabbits and healthy humans.**
Methods **Tissue blood velocity in the ONH and posterior choroid was measured using the laser speckle method as normalized blur (NB_{ONH} and NB_{CH}) in 28 anaesthetized albino rabbits. NB and intraocular pressure (IOP) in both eyes were measured for 180 min after unilateral single instillation of 5% phenylephrine and contralateral physiological saline as a control. In 11 normal volunteers aged 26.0 ± 2.7 years, NB_{ONH} was measured for 180 min after unilateral three drops of 5% phenylephrine and contralateral physiological saline in a double-masked manner. In the other 17 normal volunteers aged 25.5 ± 2.4 years, blood velocity and blood flow in a major branch of the central retinal artery were measured using the laser Doppler blood flow metre and IOP and systemic circulatory parameters were monitored in similar fashion. Analysis of variance was applied for intergroup comparisons.**
Results **NB_{ONH} and NB_{CH} decreased significantly only in the phenylephrine-treated eyes in rabbits by maximum of 9 and 20%, respectively ($P = 0.0046$ and 0.0009), despite IOP decrease. In humans, NB_{ONH} decreased significant by maximum of 13% ($P = 0.0047$) and resistive index in the retinal arteries increased by 10% ($P = 0.0067$) unilaterally; whereas IOP, diameter, blood velocity, or blood flow of the arteries was not significantly changed.**
Conclusion **Topical phenylephrine could exert a significant unfavourable effect on circulation in the ONH, choroidal tissue, and retinal arteries in rabbits and normal young humans.**

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Keywords: phenylephrine; circulation; blood velocity; blood flow; glaucoma

Introduction

Impaired local circulation is thought to be one of the dominant risk factors of various ocular disorders, such as ischaemic optic neuropathy or open-angle glaucoma. The circulation in the optic nerve head (ONH), posterior retina, or retrobulbar vessels is likely to be altered in glaucoma patients,[1–10] especially those with worse or deteriorating visual field damage,[5,7,10–13] which suggests that the impaired ocular circulation is one of the factors responsible for the development of glaucoma. There have been several reports that demonstrated cupping in the ONH followed decreased ocular perfusion without a high intraocular pressure (IOP) in animal experiments.[14,15] On the other hand, it is a probable speculation that drugs with vasoconstricting activities may be unfavourable to the posterior ocular tissues, especially those with pathological vulnerability; however, there has been only an *in vivo* study in humans where the effects of a topical vasoconstricting drug,

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apraclonidine, on the ONH hemodynamics were addressed.[16]

Phenylephrine, which is an alpha-1 agonist with vasoconstricting potential, is commonly used topically for mydriasis before intraocular surgeries or fundus examination. The chronic topical administration of phenylephrine produced significant vasoconstriction in retrobulbar arterioles around the ONH in rabbits[17] and we also found the ONH circulation was decreased in monkeys *in vivo*.[18]

The aim of this study is to further investigate the effect of phenylephrine on the circulation in the posterior parts of the eye *in vivo*, especially in humans. First, in rabbits, the possibility that topical phenylephrine affects circulation in the tissues nourished by short posterior ciliary arteries (SPCAs) not through systemic absorption, but local penetration was addressed. Then, the positive results in the rabbits prompted us to study the effects of phenylephrine on the ONH and retinal circulation in humans using noninvasive laser methods.[19–22]

Materials and methods

Animal studies

A total of 28 Japanese albino rabbits weighing 2.0–2.5 kg were anaesthetized with the intravenous injection of 0.9–1.1 g/kg urethane, and all animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The femoral artery was cannulated to monitor the mean femoral artery blood pressure (FABPm), pulse rate, arterial partial pressure of O_2 (PO_2), partial pressure of CO_2 (PCO_2), and pH. Body temperature was monitored with a rectal thermometre, and IOP was measured with a calibrated pneumatonograph. All measurements were carried out by investigators masked to the treatment.

The circulation in the ONH was measured using the laser speckle method, and the details of the method have been published previously.[19,20,23] It allows noncontact and noninvasive two-dimensional measurement of blood velocity in intraocular tissues including the ONH using laser beam and fundus camera. The laser beam (wavelength 808 nm; power 2 mW) was focused on the surface of the ONH in the largest area free of visible vessels (for NB_{ONH} measurement) or on the area of the retina just superior or inferior to the ONH without visible vessels (for NB_{CH} measurement), and the scattered light was imaged on an image sensor where the speckle pattern of the laser appeared. The difference between average speckle intensity and that of successive scans was calculated, and the ratio of the average speckle intensity to this difference was defined as normalized blur (NB). The NB value, a quantitative index of the blood velocity in the tissue, was measured every 0.125 s and the average NB level in the target area (0.42×0.42 mm on average) was calculated as $NB_{av,}$ and the successive averages of that for 30 s were refereed as NB_{ONH} or NB_{CH}.

For the analysis of NB_{ONH}, mydriasis was achieved with topical 0.4% tropicamide (Mydrin M; Santen Pharmaceutical, Osaka) in both eyes of 12 rabbits and they were kept at rest for 30 min. A 20 μl drop of 5% phenylephrine hydrochloride was instilled into one randomly chosen eye, and physiological saline was instilled into the contralateral eye as a control. IOP and NB_{ONH} in both eyes and the FABPm and pulse rate were measured before and 30, 60, 90, 120, 150, and 180 min after the phenylephrine instillation, as reported previously[19,20,24–30] by a masked investigator (JT). PO_2, PCO_2, pH, and body temperature were checked before and 90 and 180 min after the instillation.

For the analysis in the choroid, 16 rabbits were anaesthetized and the drug was topically instilled in the same procedure. Because of the difficulties in the identification of the same target area in the choroid, NB_{CH} was measured in one eye of a rabbit anaesthetized and fixed throughout the experiment as described previously.[20,28,31–33] A drop of phenylephrine was instilled unilaterally in eight rabbits, whereas the other eyes were left untreated. IOP and NB_{CH} in the phenylephrine-treated eyes were measured before and 30, 60, 90, 120, 150, and 180 min after the drug instillation, and the other eight rabbits were treated with physiological saline unilaterally and the measurements were carried out in the identical manner as controls. The measurements were also carried out by an investigator (JT) masked to the drug treatment.

Human studies

A group of 11 normal volunteers aged 23–32 (mean ± SD = 26.0 ± 2.7) years with refractive errors −7.5 to −0.75 D (mean ± SD = −4.6 ± 2.5) and another group of 17 normal volunteers aged 23–32 (mean ± SD = 25.5 ± 2.4) years with refractive errors −7.5 to 0 D (mean ± SD = −3.8 ± 2.7) were recruited with paper-based informed consent. Their normal optic disc and normal visual field were confirmed by slit lamp, fundus examination, and automated visual field test, and one who had any ocular or systemic history that could affect the current study was excluded by a careful interview. The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of University of Tokyo Graduate School of Medicine. No one was a habitual smoker or drinker of caffeine or alcohol, and physical exercise was prohibited during the experiment day.

Every subject was administered with topical 0.4% tropicamide into both eyes 30 min before the following measurements, and three doses of 20 μl 5% phenylephrine hydrochloride were instilled into one randomly chosen eye at 15 min intervals from 0900 hours. Physiological saline was instilled in the same manner into the contralateral eye as a control in a single-masked manner.

In the first group of 11 subjects, NB_{ONH} was measured just before the phenylephrine instillation and 45, 90, 135, and 180 min after the last instillation by masked investigators. The NB_{ONH} was measured using the modified apparatus used in the rabbit experiments as described previously.[28,29,34–36] The average NB level for 2–3 heart beats was referred to as the NB_{ONH}. The maximum exposure of the retina with the present apparatus was within the permissible limits of the American National Standard Institute.[37]

In the other 17 subjects, diameter of a temporal branch of the central retinal artery (CRA) and the blood velocity and blood flow through it were measured similarly before the phenylephrine instillation and 45, 90, 135, and 180 min after the last instillation . IOP, mean arterial blood pressure, and pulse rate were also monitored. IOP was measured by the Goldmann applanation tonometry twice at each time point and the mean value was adopted. Arterial blood pressure and pulse rate were measured using an automated sphygmomanometer.

Diameter, blood velocity, and blood flow of the retinal arteries were measured by the laser blood flow metre model CLBF100 (Canon, Tokyo).[22] The measurement sites were relatively straight segments of the temporal branch of the CRA that were located mostly between the disc margin and the first bifurcation, and sufficiently apart from other vessels. The instrument was equipped with an internal fixation target and an automatic vessel tracking system, and a diode laser (wavelength 675 nm) was used for the measurement. The Doppler-shifted light scattered from the flowing blood cells in the target vessel was detected in two directions. The diameter of the target vessel was measured using a laser tracking stripe oriented perpendicular to the vessel and corrected by the axial length of the eye measured and input beforehand and the refractive error was measured by the apparatus itself. The velocity, V_{max} and V_{min}, and blood flow were calculated simultaneously in the measurement. Blood flow was calculated as $V_{av} \times \text{area}/2$, where V_{av} is time average of the centerline blood velocity during a cardiac cycle and area is time average cross-sectional area at the measurement site of the target vessel according to its diameter.

Resistive index (RI) was calculated afterwards by following the formula: $RI = (V_{max} + V_{min})/V_{max}$.

A paired *t*-test was used to compare the measured values between both eyes in the same animals or humans obtained at the same time points, and an analysis of variance (ANOVA) was applied for intergroup comparison of the data obtained with sequential measurements. $P < 0.05$ was considered statistically significant.

Results

A problem in the application of the laser speckle method to the measurement of tissue blood flow is that site-to-site variations in the laser light-scattering properties of the tissues may affect the laser speckle phenomenon and obtained NB values and that properties varied among kinds of tissues or even among sites of the same tissue. Thus, NB values should not be directly compared with different tissues, such as the ONH and choroid, animals, subjects, or with right and left eyes in the absolute values. Only the NB values obtained by repeated measurements in the same area of the target should be compared as relative values in ratio or difference format. The measurement of NB values was repeated just in the same site of the target tissues using the visible surface vessels as markers, and it was confirmed in each experiment that there was no significant difference in NB values obtained preinstillation period between both eyes ($P > 0.05$). The successive values were statistically analysed in terms of difference in NB (ΔNB) between preinstillation and each time point after the drug instillation. Blood flow and RI calculated using the results of the laser Doppler blood flow metre were analysed in the same fashion.

In the rabbits, NB_{ONH} showed a maximum decrease by 9% compared to that in the preinstillation period in the phenylephrine-treated eyes at 60 min after the drug instillation ($P = 0.0004$). No such significant change was seen in the contralateral control eyes ($P > 0.14$). There were significant differences in the changes in NB_{ONH} from the baseline (ΔNB_{ONH}) between the phenylephrine-treated eyes and the contralateral control eyes ($P = 0.0046$, ANOVA) with pointwise significant inter-eye differences at 30–90 min after Bonferroni's correction ($P = 0.034$, 0.0058, and 0.0078, respectively) (Figure 1). NB_{CH} showed a maximum decrease by 20% compared to the preinstillation period in the phenylephrine-treated eyes at 60 min after the instillation ($P = 0.0011$), and there were significant inter-eye differences ($P = 0.0009$, ANOVA) in the changes in NB_{CH} from the baseline (ΔNB_{CH}) with pointwise significant inter-eye differences at 60 and 120 min after Bonferroni's correction ($P = 0.016$, 0.018 and 0.040, respectively) (Figure 2).

The IOP significantly decreased at 90 min after phenylephrine instillation by 11% compared to the

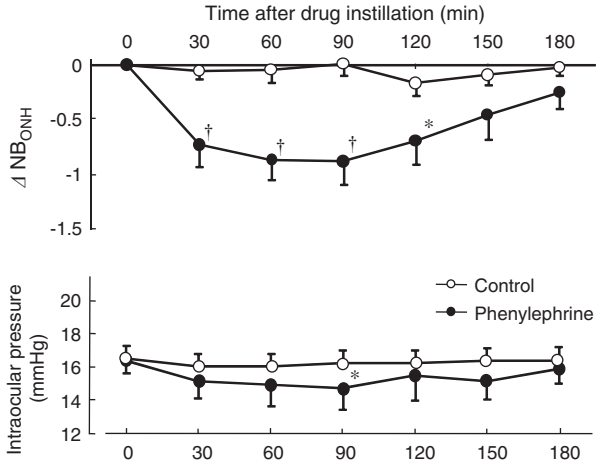

Figure 1 Changes of NB_{ONH} and intraocular pressure in rabbits. Values are mean ± SEM ($n = 12$). *Paired t-test, $P < 0.05$. †Paired t-test, $P < 0.05$ after Bonferroni's correction ($P = 0.0046$, ANOVA; NB_{ONH}).

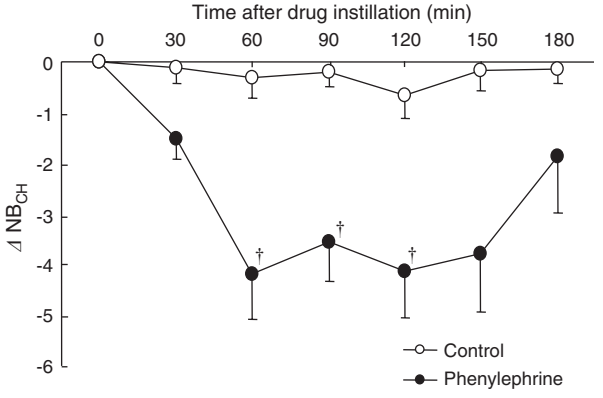

Figure 2 Changes of NB_{CH} in rabbits. Values are mean ± SEM ($n = 8$). *Paired t-test, $P < 0.05$. †Paired t-test, $P < 0.05$ after Bonferroni's correction ($P = 0.00091$, ANOVA).

preinstillation period in the phenylephrine-treated eyes ($P = 0.041$), though the inter-eye difference was not significant ($P = 0.16$, ANOVA) (Figure 1). PCO_2 declined slightly and gradually during the experimental period ($P = 0.00022$), whereas other systemic parameters, FABPm, pulse rate, PO_2, pH, and body temperature remained unchanged ($P > 0.05$).

In the human subjects, the NB_{ONH} showed a maximum decrease by 13% compared to that in the preinstillation period in the phenylephrine-treated eyes at 135 min after the drug instillation ($P = 0.0057$). There were significant differences in the changes in NB_{ONH} from the baseline (ΔNB_{ONH}) between the phenylephrine-treated eyes and the contralateral control eyes ($P = 0.0047$, ANOVA) with significant inter-eye differences at 90 and 135 min after the drug instillation after Bonferroni's correction ($P = 0.046$ and 0.032, respectively) (Figure 3).

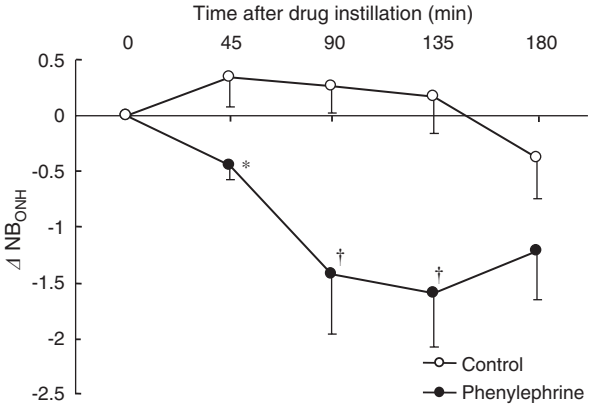

Figure 3 Changes of NB_{ONH} in humans. Values are mean ± SEM ($n = 11$). *Paired t-test, $P < 0.05$. †Paired t-test, $P < 0.05$ after Bonferroni's correction ($P = 0.0047$, ANOVA).

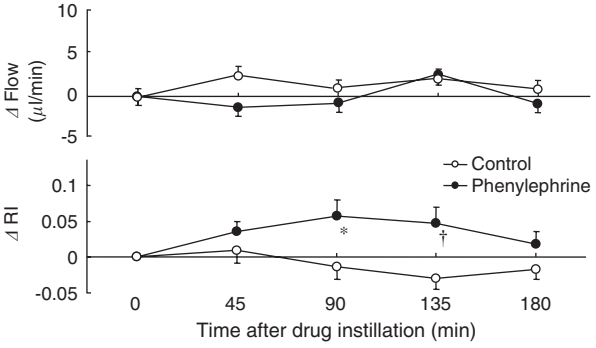

Figure 4 Changes of blood flow in the retinal arteries in humans. Values are mean ± SEM ($n = 17$). *Paired t-test, $P < 0.05$. †Paired t-test, $P < 0.05$ after Bonferroni's correction ($P = 0.0067$, ANOVA; Δ RI).

In the temporal branch of the CRA, the diameter or blood flow did not show any significant change in either the phenylephrine-treated or the contralateral control eyes ($P > 0.1$). However, RI was significantly increased at 45–135 min after the instillation of phenylephrine ($P = 0.028$, 0.023 and 0.048, respectively) with a maximum increase by 10% at 90 min after the instillation compared to the preinstillation measurement only in the phenylephrine-treated eyes. There were significant differences in the changes in RI from the baseline (ΔRI) between the phenylephrine-treated eyes and the contralateral control eyes ($P = 0.0067$, ANOVA) with significant inter-eye differences at 135 min after the drug instillation after Bonferroni's correction ($P = 0.030$; Figure 4).

The IOP in both eyes and the mean arterial blood pressure remained unchanged after the drug instillation ($P > 0.1$), though a significant but slight decline was found in the pulse rate at 180 min after the drug instillation after Bonferroni's correction ($P = 0.0082$; Figure 5).

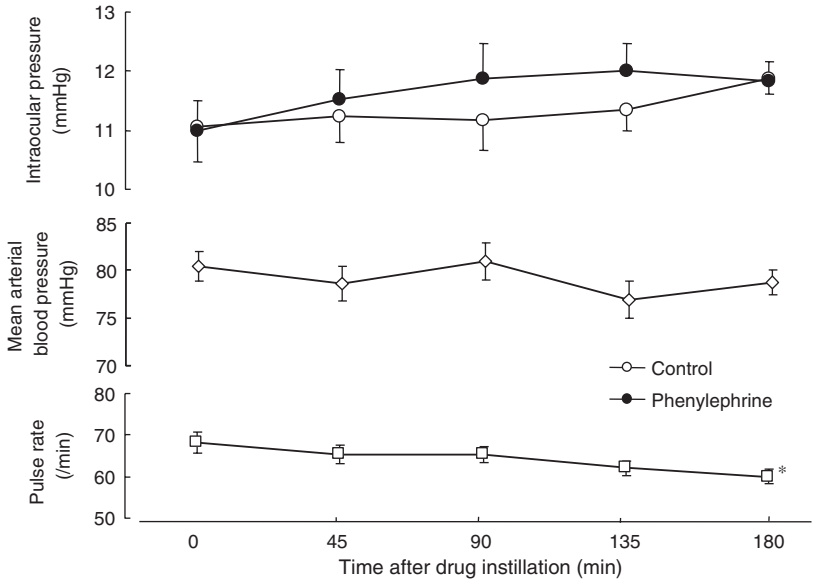

Figure 5 Changes of intraocular pressure, arterial blood pressure, and pulse rate in humans. Values are mean ± SEM ($n = 17$). *Paired *t*-test, $P < 0.05$ after Bonferroni's correction.

Discussion

The results of the laser speckle method[19,20,23] are well correlated with the blood flow determined by the micro sphere technique in the retina or uveal tissue[19,20,31,38] or the hydrogen gas clearance method in the ONH.[28,30,39] The coefficients of intrasession reproducibility of the NB measurements were reported as 7.0–7.5% for NB_{ONH} and NB_{CH} in rabbits[20] and 12% for NB_{ONH} in humans.[34] Laser Doppler velocimetry also shows a reasonable reproducibility, and its coefficients of variation for the blood flow in the retinal arteries were reported as 14% in humans.[22] Thus, the degree of change in the NB_{ONH}, $NB_{CH,}$ or RI observed in this study after phenylephrine instillation were thought to be pharmacologically significant.

The circulation in the retina or choroid, determined with the microsphere technique, was reportedly not significantly affected after the single instillation of 1% epinephrine in monkeys[40] but significantly decreased after multiple instillation of 4% epinephrine in aphakic rabbit eyes.[41] Significant vasoconstriction in retrobulbar arterioles was observed in rabbits after daily topical 2.5% phenylephrine[17] and our previous study on monkeys found the decreased ONH circulation *in vivo*;[18] however, human retinal macular leukocyte velocity was reportedly not significantly affected by topical phenylephrine.[42]

Apraclonidine is another clinically used topical drug with possible vasoconstricting effect. It was reported that a single instillation of apraclonidine was followed by the decreased blood velocities and increased resistive indices in the ophthalmic artery as determined by the Doppler ultrasonography,[43,44] whereas blood flow in the ONH or peripapillary retina measured by scanning laser Doppler flowmetry was little affected.[16] In another study assessing fundus pulsations, topical clonidine or dipivefrin significantly decreased fundus pulsations in the macula and the optic disc.[45]

In the present study in rabbits, the ipsilateral significant decrease of both NB_{ONH} by maximum of 9% and NB_{CH} by maximum of 20% in the phenylephrine-treated eyes was evident at 30 min after the instillation and continued over an hour, whereas no significant change was found in the contralateral eyes. The IOP showed a tendency to decrease in the phenylephrine-treated eyes, as previously studied in rabbits,[46] whereas the blood pressure remained unchanged. Thus, the ocular perfusion pressure in those eyes should be somewhat increased compared with the corresponding control eyes. The current experiment clearly demonstrated that topical phenylephrine unfavourably affect the choroidal and ONH circulation nourished by SPCAs by its local effects, but not systemic effects in rabbits.

Mizuno *et al*[36] demonstrated in monkey eyes that topically instilled nipradilol reached the ipsilateral retrobulbar space adjacent to the optic nerve insertion at a pharmacologically active concentration using radioactive agent, and Ishii *et al*[47] reported similar retrobulbar distribution of topical iganidipine in rabbits. Taken together with those reports, the results of the present rabbit study may indicate that topically instilled

phenylephrine reached the ipsilateral retrobulbar tissues by local penetration and exerted a vasoconstrictive effect on the SPCAs or its branch arteries only in the instilled side, which supply blood flow to the ONH and posterior choroid in rabbits.[48] This speculation is compatible with the current finding, but also those of Sugiyama *et al*[17] that showed significant constriction of the arterioles supplying ONH in rabbits after chronic topical administration of phenylephrine by microvascular casting technique, and the finding in monkeys by Takayama *et al*[18] that NB_{ONH} significantly decreased ipsilaterally by 26% after 7 days twice-daily topical administration of phenylephrine.

In the present human study, we first found that topical phenylephrine made the ipsilateral significant decrease in the NB_{ONH} 90–135 min by maximum of 13% and significant increase in RI at 135 min by 10% after the instillation. The insignificant effect of phenylephrine on the IOP of normal human subjects found in this study is consistent with previous results in human, which reported that topical 2.5 or 10% phenylephrine had little effect on aqueous flow or IOP in normal human subjects.[49,50] In that case, the ocular perfusion pressure was not significantly affected by the instillation of phenylephrine together with the unaffected arterial blood pressure.

The blood supply to the ONH is not the same between humans and rabbits; however, the SPCAs supply blood flow of the ONH both in rabbits and humans[48,51,52] and effects of topically instilled drugs on the ONH circulation were reportedly similar in rabbits and humans. Several studies have found increased circulation in the ONH in rabbits[25–27] and in humans,[36] and that in the posterior retina or choroid in humans[53–55] after the topical administration of beta antagonists. Favourable effects on circulation in the ONH were also found for topical prostaglandin analogues in experimental animals[29] and in humans,[29,56,57] and that in the retinachoroid in humans.[57] Those drugs reportedly affected only the ipsilateral instillation side, which suggested that the drug locally distributed to the posterior segment of the eye at pharmacologically active levels.

Thus, the decrease in the NB_{ONH} and increase in RI in the retinal arteries in humans after phenylephrine instillation found in this study may suggest that the topically instilled phenylephrine locally distributed to the ipsilateral retrobulbar tissues around the SPCAs and retinal arteries at a pharmacologically active level, and that is compatible with the decreased blood velocities and increased resistive indices found in the ophthalmic artery after instillation of apraclonidine[43,44] and with the decreased fundus pulsation in the macula and the optic disc after instillation of clonidine.[45] On the other hand, it is not clear whether the increased RI in the temporal retinal artery is attributed to direct effect of phenylephrine on the retinal branch arteries or central retinal artery. No significant change in the blood flow despite the increased RI may be explained by autoregulatory mechanism of retinal circulation.

The decreased NB_{ONH} and increased RI recovered to the preinstillation level within 180 min after the drug instillation, and it is not clear to what extent the transient decrease in the ONH circulation will actually have a clinical impact. Phenylephrine, often instilled to patients in routine clinical situations, especially for the maintenance of mydriasis during intraocular surgeries would have little influence in normal young subjects; however, that might have significant effect in older subjects with local circulatory disorders or with the ONH already damaged by diseases, such as glaucoma or ischaemic optic neuropathy. Actually, there was a report on acute worsening of visual function in four patients with nonarteritic ischaemic optic neuropathy at 45 min to 12 h after instillation of one drop of 2.5% phenylephrine and 0.5–1% tropicamide for dilated funduscopic examination.[58] Further investigation of phenylephrine effects is required in those with preexisting impairment of local circulation.

In summary, we confirmed that topically instilled 5% phenylephrine hydrochloride significantly decreased the blood velocity in the choroid and ONH mainly nourished by SPCAs by local penetration in rabbits, and first demonstrated that blood velocity in the ONH was significantly decreased and RI in the retinal arteries was significantly increased after topical phenylephrine in normal young humans in this study. The current finding indicates that topical vasoconstricting agents including phenylephrine can unfavourably influence the ONH and retinal circulation not only in experimental animals, but also in humans.

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