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Effect of Topical Dorzolamide on Tissue Circulation in the Rabbit Optic Nerve Head

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Purpose: To examine the effect of 1% topical dorzolamide on tissue circulation in the optic nerve head (ONH) of Dutch rabbits.

Methods: A laser speckle tissue circulation analyzer was used. One eye of each rabbit received 1% topical dorzolamide twice daily for 20 days, and the fellow eye received the vehicle in a masked, randomized manner. Intraocular pressure (IOP) was measured every 5 days. The normalized blur (NB) value, a quantitative index of tissue blood flow velocity in the ONH, was measured before treatment and 2 hours after the last instillation on the 20th day.

Results: The IOP was lowered by about 2 mm Hg only in the dorzolamide-treated eyes ($P < .01$). The NB value showed no significant change in either dorzolamide-treated or vehicle-treated eyes.

Conclusions: Long-term topical dorzolamide does not affect the ONH tissue circulation in dorzolamide- and vehicle-treated eyes of Dutch rabbits. **Jpn J Ophthalmol 1999;43:386–391** © 1999 Japanese Ophthalmological Society

Key Words: Dorzolamide, laser speckle phenomenon, optic nerve head, rabbit eye, tissue circulation.

Introduction

Carbonic anhydrase inhibitors, which reduce aqueous production with a corresponding decrease in intraocular pressure (IOP), have been used systemically for the treatment of glaucoma.[1] Acetazolamide, a carbonic anhydrase inhibitor, is known to increase the cerebral blood flow when administered orally or intravenously.[2,3] Studies of the effect of 1000 mg acetazolamide on the cerebral circulation in the human eye have shown a 38% increase in cerebral blood flow after an oral dose[2] and a 70% increase after the same dose given intravenously.[3] This increase in cerebral blood flow was due mainly to cerebral vascular dilatation. Acetazolamide was also found to increase the partial pressure of carbon dioxide (P_{CO2}) and to reduce the pH in the cerebral extracellular fluid.[4] This suggests that the direct effect of acetazolamide on tissue P_{CO2} and on pH is to act as a potent stimulus for vasodilatation.

In the eye of the monkey, increasing P_{CO2} in the retinal circulation has also been shown to produce retinal vasodilatation.[5] Using laser Doppler velocimetry and computerized digital image analysis of monochromatic fundus photographs, it was determined that retinal blood flow in the human eye was significantly increased (37%) after intravenous administration of 500 mg acetazolamide.[6] In addition to the metabolic effects on cerebral circulation, acetazolamide reduced the IOP, thereby affecting retinal perfusion pressure.

To alleviate systemic side effects of oral carbonic anhydrase inhibitors,[7] dorzolamide hydrochloride, a topical carbonic anhydrase inhibitor, was recently developed for the treatment of glaucoma.[8,9] There have been several reports of the effect of dorzolamide on blood flow in the retina,[10–12] choroid,[13] or ophthalmic artery.[10] However, relatively few reports are available on its effect on the circulation in the optic nerve head (ONH) tissue. Sugrue[14] instilled 2% dorzolamide either 1 hour before or twice daily for 10 days in albino rabbits. He could not find any significant change in blood flow as determined by the

Received: June 10, 1998
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Published by Elsevier Science Inc.
0021-5155/99/$–see front matter
PII S0021-5155(99)00093-3

radioactive microsphere method in both regimens. However, 15 μm radioactive microspheres were used, and the number of spheres trapped in the optic nerve was not sufficient for the detection of a small change in the ONH blood flow.[15,16]

Using scanning laser ophthalmoscopy, Harris et al[10] reported that two drops of topical 2% dorzolamide caused a significant increase in blood velocity in the superficial optic nerve capillaries of young healthy individuals. On the other hand, Pillunat et al[17] reported that administration of dorzolamide drops for three days, three times per day in human eyes did not cause any significant change in ONH blood flow as measured by laser Doppler flowmetry. Scanning laser ophthalmoscopy used by Harris et al[10] and laser Doppler flowmetry used by Pillunat et al[17] should be much more sensitive in detecting changes in blood flow velocity in the ONH, but their results did not describe the effects of long-term dorzolamide treatment, where concentrations in ONH tissue might accumulate to a much higher level than that after two or three times of instillation per day. If a pharmacologically effective quantity of dorzolamide reaches the ONH through systemic absorption or by direct drug infiltration, as suggested for phenylephrine,[18] the ONH tissue blood flow may be significantly affected by topical dorzolamide. Previous studies have indicated that a compromise of the tissue circulation in the ONH may play a causal role in glaucomatous injury in the ONH, although IOP has been consistently found to be one of the most important risk factors in the development of open-angle glaucoma.[19–21] Therefore, the possible effects of dorzolamide on ONH tissue circulation are of great clinical importance. The clinical finding that three drops per day for 3 days of dorzolamide in normal human eyes increased perimetric light sensitivity[22] may be related to the effects of dorzolamide on the ONH tissue circulation.

The laser speckle method, by which a quantitative index called the normalized blur (NB) value can be obtained, was used to measure the in vivo tissue blood flow velocity in an area of 0.42 × 0.42 mm in the ONH with a reproducibility of about 10%.[23] We have recently found that twice daily, for 20 days of 0.5% timolol,[24] 0.5% betaxolol,[25] or 2% carteolol[26] caused a significant effect on the ONH tissue blood velocity in albino rabbits through local and/or systemic effects.

The rabbit ONH vasculature has some features similar to that of humans and primates; the principal blood supply of the rabbit ONH is derived from the short posterior ciliary arteries by the arterial circle.[27] In the present experiment, we studied the effects of twice-daily, 20-day instillation of 1% dorzolamide on the ONH tissue circulation in rabbit eyes using the same method. The concentration of 1% was selected because a previous study, which tested concentrations of 0.2%, 0.5%, 1%, and 2% in Japanese patients, showed that concentrations of 0.5 and 1% were the most effective in terms of efficacy and acceptability to patients.[28]

Materials and Methods

Laser Speckle Tissue Circulation Analyzer

The ONH tissue circulation was evaluated with a laser speckle tissue circulation analyzer, which has been described in detail elsewhere.[23] The apparatus consists of a fundus camera (TRC-WT3; Topcon, Tokyo) equipped with a diode laser (wavelength 808 nm) and an image sensor (100 × 100 pixels, Basis type; Canon, Tokyo). A halogen lamp illuminates the fundus where the laser beam is focused. The scattered laser light is imaged onto the image sensor that corresponds to a field of 0.62 × 0.62 mm in the rabbit ONH, where a speckle pattern appears. The difference between the average of the speckle intensity (I_{mean}) and the speckle intensity for successive scannings of the image speckles at the pixels on the sensor plane was calculated. The ratio of the I_{mean} of this difference was defined as the NB value. Normalized blur is nearly equivalent to the reciprocal of speckle contrast described by Fercher and Briers[29,30] and is thought to be primarily indicative of tissue blood velocity. The results are displayed in a color graphic showing the two-dimensional variation of the NB level over the field of interest. The average of the NB levels in an area free of visible surface vessels in the measured field in the ONH was expressed as NB_{av}. The coefficient of reproducibility was approximately 10% for 5-minute or 24-hour interval in vivo measurements of the NB_{av} in a square area of 0.42 × 0.42 mm free of visible surface vessels in the rabbit ONH (70 × 70 pixels on the sensor plane).[23]

Drug

A 1% ophthalmic solution of dorzolamide hydrochloride and its vehicle were kindly supplied by Banyu Pharmaceutical Company (Tokyo).

NB_{av} Measurements in ONH

Eleven Dutch rabbits weighing 1.5–1.8 kg were used and handled in accordance with the ARVO Resolution on the Use of Animals in Research. The

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animals were acclimated to a light schedule of alternating 12-hour periods of light and dark (12L:12D; light on at 4 A.M.) for at least 3 weeks before use. After dilating the pupil with one drop of Mydrin M (0.4% tropicamide; Santen Pharmaceutical, Osaka), the image speckles from the largest square field in the ONH free of visible surface vessels were recorded to measure the NB_{av} value in the ONH tissue ($NB_{av(ONH)}$). Color fundus photographs were taken to record the site of the NB measurements using visible surface vessels near the measurement field as markers to identify the tissue sites.

Experimental Protocol

The IOP was measured in both eyes with a calibrated applanation pneumotonometer after instillation of topical anesthesia (0.4% oxybuprocaine hydrochloride) at 8 P.M. under dim light. General anesthesia was induced by an intravenous injection of 15 mg/kg pentobarbital sodium. The pupil was dilated as described. Fifteen minutes after induction of general anesthesia, the $NB_{av(ONH)}$ was measured in both eyes as described. The average of six measurements obtained at 1-minute intervals was adopted as the initial value. Color fundus photographs were taken to record the site of NB measurement.

From the next day, 1 eye of each animal received 20 μL of 1% dorzolamide, and the fellow eye received the vehicle twice daily (6 A.M. and 6 P.M.) for 20 days in a masked manner (n = 11). During the treatment period, the light schedule was the same as described above, and the IOP was measured under topical anesthesia in both eyes at 8 P.M. on the 5th, 10th, 15th, and 20th day under dim light. On the 20th day, after measuring the IOP under topical anesthesia, general anesthesia was induced. The $NB_{av(ONH)}$ at the same site of ONH tissue was measured again as described, in both eyes. All measurements were carried out by investigators unaware of the treatment given to the animals.

Calculations and Statistical Analysis

The results are presented as the mean ± standard error of the mean (SEM). Paired Student's *t*-tests and analysis of variance (ANOVA) for repeated measurements were used to evaluate statistical significance. Significance levels of $P < .05$ were considered statistically significant.

Results

The IOP in the dorzolamide-treated eyes was reduced significantly, when compared with that in the contralateral eyes, with a maximum reduction of 2.2 mm Hg on the 15th day of treatment (ANOVA on repeated measurements, $P < .01$). The IOP in the contralateral control eye showed no significant change from its baseline value throughout the study period (Table 1). Before the instillation, no significant bilateral difference in the $NB_{av(ONH)}$ was seen (paired *t*-test, $P = .386$) (Table 2). The $NB_{av(ONH)}$ showed no significant difference from the baseline in either eye after the 20-day treatment ($P = .900$ and $P = .358$), and no significant bilateral difference in $NB_{av(ONH)}$ was found (paired *t*-test, $P = .918$). The $NB_{av(ONH)}$ values before and after completion of the test were not significantly different for each pair of eyes ($P = .355$).

Discussion

Method of Measurement

According to Koelle et al,[31] the penetration depth of the near-IR laser (wavelength 811 nm) in the cat optic nerve exceeds 1 mm. Thus, using the present apparatus, the effective depth of sampling in the ONH tissue should be greater than 1 mm. The NB_{av}

Table 1. Effect of 1% Dorzolamide on Intraocular Pressure (mm Hg)

		After			
	Before Treatment	5 Days	10 Days	15 Days	20 Days
Dorzolamide	27.0 ± 1.2	25.2 ± 1.0	25.6 ± 1.0	24.8 ± 1.1	25.3 ± 1.4
Control	27.4 ± 1.2	26.7 ± 1.1	27.4 ± 0.9	27.0 ± 1	27.5 ± 1.3

Values represent mean ± SEM in 11 rabbits. Dorzolamide indicates eyes treated with 0.5% dorzolamide. Control indicates contralateral control eyes instilled with its vehicle. Topical dorzolamide significantly reduced intraocular pressure only in dorzolamide-treated eyes (analysis of variance on repeated measurements, $P < .01$).

Difference between dorzolamide and control eyes was significant on 5, 10, 15, and 20 days (paired *t*-test, $P < .05$–$.01$).

Table 2. $NB_{av(ONH)}$ Before and After 20-day Instillation of Dorzolamide

	Before	20 Days After	Δ
Dorzolamide	12.3 ± 0.2	12.3 ± 0.2	0.0 ± 0.2
Control	13.0 ± 0.9	12.3 ± 0.3	−0.7 ± 0.8

Values indicate mean ± SEM in 11 rabbits. Dorzolamide indicates eyes instilled with 1% dorzolamide. Control indicates contralateral control eyes instilled with its vehicle. Δ indicates difference between value on 20th day and that before treatment.

is primarily a quantitative index of the velocity of blood cells, but it was found that this parameter is also affected by the number of blood cells in the measured tissue.[32] The NB_{av} obtained from the rabbit ONH showed a high and significant correlation with the change in the ocular perfusion pressure (OPP) after injection of a lethal dose of pentobarbital.[24] If the change in the OPP occurs faster than any autoregulatory change in vascular resistance, as would be expected after injection of a lethal dose of pentobarbital, then the blood flow rate is expected to parallel the OPP. Therefore, a significant correlation between the NB_{av} obtained from the rabbit ONH and the OPP after injection of a lethal dose of pentobarbital suggests not only that the decrease in OPP paralleled the NB_{av}, but also that the NB_{av} correlates with the blood flow rate in the ONH tissue under these conditions.

Using the same type of apparatus, Sugiyama et al[33] measured the NB_{av} in the ONH of rabbits. The ONH tissue blood flow rate was determined by the hydrogen gas clearance method in the same rabbit before and after inhalation of 10% CO_2 or intravenous injection of a small amount (10^{-10} M/kg) of endothelin-1 (ET-1). A significant correlation was found between the relative change in NB_{av} and in the ONH tissue blood flow rate determined by the hydrogen gas clearance method. These results suggest that the NB_{av} obtained in the ONH correlates with the ONH tissue blood flow rate at least under these conditions.

Effect of Topical Dorzolamide on ONH Tissue Circulation

In the present study, $NB_{av(ONH)}$ showed little change after 20 days of twice-daily instillation of dorzolamide. In a separate experiment, the $NB_{av(ONH)}$ measurements were made in the same region of the ONH in 15 untreated rabbit eyes at intervals of 3 weeks.[24] No significant difference was observed between the two values, and the standard deviation of the difference between the two values was 10.5% of the mean value of the $NB_{av(ONH)}$. Thus, with a sample size of 11, a change in the $NB_{av(ONH)}$ of 9.5% or greater would be detected ($\alpha = 0.05$, $\beta = 0.8$).[34]

In our previous report, where the OPP (calculated as blood pressure minus IOP) was reduced by increasing the IOP, NB_{av} obtained from ONH tissue showed autoregulation after the acute reduction in the OPP.[23] In the present experiment, the OPP could not be calculated because the blood pressure was not measured. However, the bilateral difference in IOP in the dorzolamide-treated eyes should be the same as the difference in OPP. In the present study, the significant decrease in the average IOP of about 2 mm Hg was observed in the dorzolamide-treated eyes. In spite of this change, no significant change in $NB_{av(ONH)}$ was observed. This may be due to the autoregulatory capacity of the ONH vasculature, which would tend to keep $NB_{av(ONH)}$ unchanged despite small fluctuations in the IOP.[23] Using the blue field simulation technique, Grunwald and Zinn[35] reported that an oral administration of 500 mg of acetazolamide had no significant effect on human retinal macular leukocyte velocity. They also reported that a single dose of topical dorzolamide caused no significant change in the blood velocity or flow rate in a main temporal vein of subjects, as determined with laser Doppler velocimetry and monochromatic fundus photography.[11] Furthermore, three times per day instillation of dorzolamide for three days did not cause any significant change in ONH blood flow[15] or peripapillary retinal blood flow,[12] as determined by laser Doppler flowmetry. The present result is not inconsistent with these findings.

On the other hand, Rassam et al[6] reported that 500 mg of acetazolamide administered intravenously caused a significant increase in retinal blood flow determined by laser Doppler velocimetry. The difference in the results between this study and ours could be due to the much higher concentrations of drugs achieved by Rassam et al,[6] who injected an intravenous bolus of 500 mg of acetazolamide. As they suggested, the retinal blood flow changes in their study may be due to the systemic metabolic effects of acetazolamide. Because of the much higher drug concentrations achieved by them, such effects would be much greater than those seen in previous studies or in the current study. Using scanning laser ophthalmoscopy and color Doppler imaging, Harris et al[10] reported that two drops of dorzolamide hastened the retinal arteriovenous passage of fluorescein dye, accelerated the capillary dye transit in the macula and optic nerve, and left unaltered the blood velocity or resistance index in any retrobulbar vessel. The dif-

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ference between these results and ours could be due to: (1) differences in ONH vasculature between rabbits and humans; (2) the use of pentobarbital anesthesia in rabbits might relax the vascular tone[36] and mask the small vascular relaxing effect of dorzolamide observed in humans; or (3) a difference in the blood flow parameters measured by the two methods, ie, the effective depth of sampling in $NB_{av(ONH)}$ measurements is thought to be about 1 mm from the surface of the ONH,[23] whereas the dye transit determined by Harris et al[10] correlates only with blood velocity in the superficial capillaries of the optic nerve head.

In the present study, a significant reduction in IOP was found only in the treated eye, which indicated that the level of circulating drug was too low to exert a pharmacological effect on the contralateral eye under the conditions of this experiment. Thus, the effect of dorzolamide on ONH tissue circulation, if any, would be caused by the drug that penetrated locally. According to Maren,[7] the concentration of dorzolamide in the ciliary body was 10 μmol/L (3.6 μg/g) after a single instillation of 2% dorzolamide, which exerted a 99.9% inhibition of carbonic anhydrase II and a 97% inhibition of carbonic anhydrase IV in albino rabbits.[37] Although data are not available on the dorzolamide concentration in ocular tissues of pigmented rabbits after 20 days of twice-daily instillation, it seems unlikely that dorzolamide reaches the retina at a level sufficient to exert over 99% inhibition of the carbonic anhydrase activity.[38] The results of the present study might be explained by the fact that the concentration of dorzolamide in the back of the eye, including ONH tissue, did not reach a sufficient level to exert over a 99% inhibition of carbonic anhydrase.

Because the dimensions of the human eye are greater than those of the rabbit, especially in the posterior segment of the eye, the concentration of dorzolamide in the back of the eye in humans is attributable to local penetration and is probably lower than that in rabbits. Dorzolamide concentration in the plasma after long-term topical use in human eyes is reported to be about 0.034 μmol/L and exerts no physiological effects on carbonic anhydrase inhibition.[39] The present results suggest that it would be difficult to obtain significant circulatory effects in the back of the human eye, which could be attributed to the local penetration or systemic absorption of dorzolamide after only 3 weeks of twice-daily instillation.

This work was supported in part by Grant-in-Aid for Developmental Scientific Research (B) No. 01870074 from the Ministry of Education, Science, Sports and Culture of Japan.

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