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Effect of Topical Timolol on Optic Nerve Head Circulation in the Cynomolgus Monkey

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Purpose: To evaluate the effect of topical timolol on the optic nerve head (ONH) circulation in the cynomolgus monkey.

Methods: Eight cynomolgus monkeys were used. Timolol (0.5%, 30 μL) was instilled twice daily into 1 eye and physiological saline into the other eye for 7 consecutive days. The ONH tissue blood velocity (NB_{ONH}) was determined using the laser speckle method. The determinations of NB_{ONH} were carried out before the first instillation and 4 hours after the first, the seventh and the last instillation. The IOP was measured periodically. All measurements were performed in a masked manner.

Results: Twice daily 7-day instillation of 0.5% timolol showed no significant effect on the NB_{ONH} in either eye, while the IOP was significantly decreased in both eyes.

Conclusion: In the cynomolgus monkey, the ONH circulation was not affected by 7-day twice daily topical timolol treatment. **Jpn J Ophthalmol 2000;44:630–633** © 2000 Japanese Opthalmological Society

Key Words: Cynomolgus monkey, intraocular pressure, laser speckle method, optic nerve head circulation, timolol.

Introduction

Timolol, a nonselective β-adrenergic antagonist, has probably been the most widely used glaucoma medication. β-Adrenergic receptors generally mediate relaxation of vascular smooth muscle and systemic β-adrenergic antagonists induce vasoconstriction in various tissues.[1,2] Whether or not this agent has any effect on ocular blood flow has been intensively studied, but is still controversial.[3–7] The optic nerve head (ONH) has been considered the tissue where glaucomatous damage takes place, and the effect of the topical timolol on the ONH circulation is of clinical importance. Yoshida et al[4] reported that a single instillation of timolol showed no significant effect on capillary blood flow velocity in the ONH measured by laser Doppler velocimetry. According to Jay et al,[5] the blood flow in the optic nerve determined by the radioactive microsphere method was not affected in phakic rabbit eyes when timolol was applied topically at a rate of eight drops per 7.5 minutes. Laser doppler velocimetry used by Yoshida et al[4] should be much more sensitive in detecting small changes in blood flow velocity in the ONH.[8] However, the effects of long-term timolol treatment, by which the concentration of timolol in ONH tissue is expected to be elevated to a much higher level than after a single instillation, were not described in this study. In case of long-term instillation, Tamaki et al[6] reported that 20-day twice daily instillation of timolol significantly increased the ONH tissue circulation as measured by the laser speckle method in rabbit eyes, but not in human eyes.[7] In the already-cited human study,[7] no significant change in either blood pressure or pulse rate was seen. Cynomolgus monkeys have an ONH structure and vasculature similar to humans,[9] but ocular or body dimensions are much smaller than those of humans. Thus, plasma concentration of timolol or local and posterior penetration of timolol should be higher in this

Received: November 29, 1999
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Published by Elsevier Science Inc.

0021-5155/00/$–see front matter
PII S0021-5155(00)00276-8

species than in humans. Moreover, the cynomolgus monkey is thought to serve as an appropriate animal model for showing if topical timolol has the potential to influence the ONH circulation. In this study, we investigated the effects of 7-day twice daily topical timolol treatment on the ONH circulation in cynomolgus monkey eyes using the laser speckle method.[6,7,10]

Materials and Methods

Determinations of Circulation in ONH Tissue

Tissue circulation in the ONH was evaluated using the laser speckle method; details of this method have been reported elsewhere.[10] An apparatus consisting of a fundus camera (TRC-WT3®; Topcon, Tokyo) equipped with a diode laser (wave length = 808 nm) was used for measurement. The scattered laser light was visualized on an image sensor (100 × 100 pixels, Basis type; Canon, Tokyo). The difference between the average of the speckle intensity (I_{mean}) and the speckle intensity for successive scanning of the image speckles at the pixels on the sensor plane was calculated, and the ratio of I_{mean} to this difference was defined as normalized blur (NB), which is thought to be indicative of tissue blood velocity.[10] In this experiment, NB determined for 5 seconds during which there was no eye movement, five times, each time after a 30-second interval, and the average of three measurements excluding the maximum and the minimum values was adopted. After dilating the pupil with one drop 0.4% tropicamide (Mydrin M®; Santen, Osaka), the image speckles from the largest square field in the ONH free of visible surface vessels were recorded to determine the NB_{ONH}. Color fundus photographs were taken to record the site of the NB_{ONH} determinations, and the visible surface vessels near the measurement field were used as markers.

Experimental Procedures

Eight adult cynomolgus monkeys (3–7 kg) were used and handled in accordance with the ARVO Resolution on the Use of Animals in Research.

At 12:00 PM on the 1st experimental day, under general anesthesia induced by ketamine hydrochloride (Ketalar®; Sankyo, Tokyo) at a dose of 8–10 mg/kg intramuscularly, the NB_{ONH} was determined as described above in both eyes. Then, IOP measurement in both eyes (baseline IOP at 12:00 PM) was performed with a calibrated applanation pneumotonometer (Alcon Labs, Fort Worth, TX, USA) after an instillation of topical anesthetics (0.4% oxybuprocaine hydrochloride, Benoxil®; Senju, Osaka). The blood pressure and pulse rate were measured simultaneously with a sphygmomanometer for an infant (SP-8800; Nihon Koden, Tokyo). The mean blood pressure (BP_m) was calculated according to the formula: $BP_m = BP_d + 1/3\ (BP_s - BP_d)$, where BP_d and BP_s are diastolic and systolic brachial blood pressure, respectively. The ocular perfusion pressure (OPP) was calculated according to the formula: $OPP = 2/3\ BP_m - IOP$. Arterial O_2 saturation (SaO_2) was checked using the pulse oxygen meter (OLV-1200; Nihon Koden, Tokyo), and body temperature was monitored with a Thermopit (IT-500M; Nipro, Osaka).

From the 2nd experimental day, 30 μL of 0.5% timolol maleate (Banyu, Tokyo) was instilled twice daily for 7 days into one randomly chosen eye and physiological saline into the other eye to serve as control.

From the 2nd to the 8th experimental day, the IOP measurement without general anesthesia was carried out at 8:00 AM under topical anesthesia before the morning timolol instillation. At 12:00 PM on the 2nd, 5th, and 8th experimental days, NB_{ONH}, IOP, blood pressure, pulse rate, SaO_2, and body temperature measurements were carried out under general anesthesia as above. All measurements were performed by an investigator masked to the treatment.

Calculation and Statistical Analysis

The results are presented as mean ± standard deviation. Paired Student *t*-test was used and correction for the number of comparisons was performed by Bonferroni's method.

Results

Parameters of the systemic condition of the monkeys during the period when the NB_{ONH} measurements were performed under general anesthesia are shown in Table 1. A significant 17% reduction compared with the baseline level in the pulse rate was observed ($P = .002–.016$). Other parameters showed no significant changes.

The IOP at 8:00 AM in the timolol- or saline-treated eyes was significantly lower than baseline IOP between the 6th and 8th experimental day ($P = .005–.045$), respectively, whereas the IOP in the timolol treated-eyes was significantly lower than that in the saline-treated eyes between the 3rd and 5th days ($P = .003–.025$)(Figure 1).

The IOP at the time of the NB_{ONH} measurement (12:00 PM) was significantly lower than the baseline levels ($P = .001–.028$) in both the timolol- and saline-treated eyes. No significant bilateral difference was

Jpn J Ophthalmol
Vol 44: 630–633, 2000

Table 1. Systemic Parameters During Experiment

Parameter	Experimental Day*			
	1	2	5	8
BP_m (mm Hg)	68.5 ± 9.27	66.4 ± 7.92	66.2 ± 10.1	70.7 ± 8.6
Pulse rate (beats/min)	171.6 ± 23.6	141.3 ± 23.9 ($P = .002$)†	142.3 ± 21.6 ($P = .008$)†	144.4 ± 17.8 ($P = .016$)†
BT (°C)	36.3 ± 0.5	36.5 ± 0.6	36.4 ± 0.5	36.3 ± 0.5
SaO_2	99.2 ± 0.3	99.0 ± 0.6	98.2 ± 0.8	99.1 ± 0.5

BP_m: Mean arterial blood pressure; BT: body temperature; SaO_2: saturation of arterial O_2.
*Values are mean ± SD (n = 8).
†*P* value by paired *t*-test with Bonferroni's correction.

seen (Figure 2A). The OPP was significantly higher than the baseline in both eyes on the 8th experimental day, showing about a 17% increase ($P = .017$ and .022) (Figure 2B).

The NB_{ONH} showed no significant change in either the timolol- or the saline-treated eyes during the experimental period (Figure 2C).

Discussion

In the present study, the pulse rate decreased by 17% and the IOP in the saline-treated eye also decreased. Thus, the concentration of timolol in the blood is considered to have been sufficiently high to cause systemic β-blocking effects. Nevertheless, the NB_{ONH} showed virtually no change at any time points throughout the experiment. The same conclusion was previously suggested by Tamaki et al[7]), who studied the effect of twice daily 3-week instillation of 0.5% timolol on the ONH circulation in the normal human eye. In their human subjects, pulse rate and blood pressure showed virtually no change, which suggests that systemic β-blocking effects were much weaker in their experiment than in the present one.

These results obtained for topical timolol in humans are different from those obtained in rabbits[6] and those for topical carteolol or betaxolol, both of which showed an increase in the NB_{ONH} effects.[7,11–13] Since carteolol or betaxolol also showed an ocular hypotensive effect comparable to that of timolol, the difference between timolol and carteolol or betaxolol is readily understandable as being due to a difference in pharmacological effects other than β-blocking

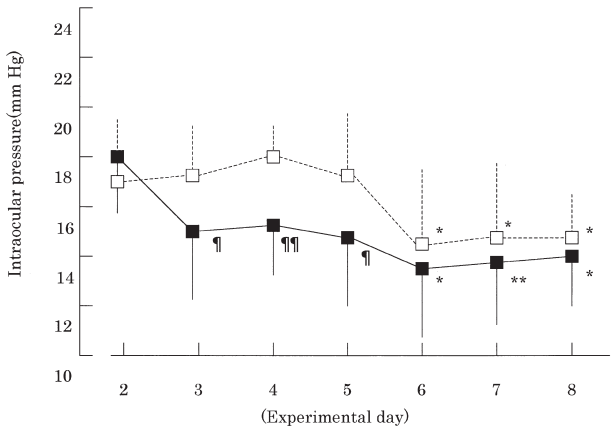

Figure 1. Time course (day) of intraocular pressure at 8:00 AM after topical instillation of timolol (■) or saline (□) in monkey eyes. Each plot represents mean value. Error bars represent standard deviation (SD). *Difference from baseline at $P < .05$ with Bonferroni's correction. **Difference from baseline at $P < .01$ with Bonferroni's correction. ¶Difference from saline-treated eyes at $P < .05$ with Bonferroni's correction. ¶¶Difference from saline-treated eyes at $P < .01$ with Bonferroni's correction.

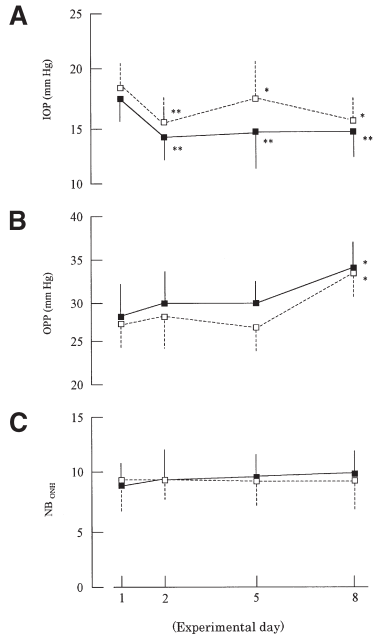

Figure 2. Intraocular pressure (IOP) (**A**), ocular perfusion pressure (OPP) (**B**) and optic nerve head tissue blood velocity (NB_{ONH}) (**C**) in timolol-treated (■) or saline-treated (□) eyes. Each plot represents mean. Error bars show standard deviation (SD). *Difference from baseline at $P < .05$ with Bonferroni's correction. *Difference from baseline at $P < .01$ with Bonferroni's correction.

effect.[7,11–13] The lack of effect of topical timolol on the NB_{ONH} observed in this experiment in spite of the adequate systemic β-blocking effect may be explained as follows: (1) The β-system is not physiologically functional in the vascular system supplying the ONH, which may be more evident in the monkey eye than in the human eye and is different from that in the rabbit eye. (2) Under the systemic conditions in which the tissue blood flow is affected, the autoregulatory mechanism usually operates in these tissues to bring about stabilization.[14–18] A reduction in the blood flow based on a decrease in the heart rate should result in an increase in the stroke volume.[14,19] The β_2 blocking agents decrease the heart rate and have a vasoconstrictive effect in the peripheral tissues,[1,2,20] thereby causing a decrease in the tissue blood flow. However, an increase in cardiac output may have masked the blood flow decreasing effect of topical timolol in the ONH tissue. (3) Grunwald[21,22] repeatedly reported that topically applied 0.5% timolol increased the centerline blood velocity through the retinal vein in human eyes and that its effect correlated with an increase in the ocular perfusion pressure (OPP) caused by IOP reduction. It may also be possible that the blood flow decreasing effect of topical timolol due to its vasoconstrictive effect was cancelled out by the blood flow increasing effect due to an increase in the OPP. This hypothesis, however, cannot explain the result obtained on the 2nd or 5th day, but only that on the 8th day when the OPP increased.

In any case, the lack of effect of topical timolol on the ONH circulation in monkey eyes despite stronger systemic β-blocking effect further supports the hypothesis that topical timolol will not show a clinically significant influence on ONH circulation.

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