

Effect of Topical Carteolol on Iridial Circulation in Pigmented Rabbit Eyes

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Abstract: The effect of a single instillation of topical carteolol on iridial tissue circulation of pigmented rabbits was studied. The blood flow rate and a quantitative index of tissue blood velocity (NB_{iris}) were measured simultaneously, using the microsphere technique and the laser speckle method, before and 2 hours after the instillation of 20 microliters of 2% carteolol or the vehicle. Consecutive changes of intraocular pressure and NB_{iris} were also studied at 30-minute intervals for 2.5 hours after a single instillation of 2% carteolol in one eye and the vehicle in the contralateral eye. To provide a control, intraocular pressure and NB_{iris} were measured according to the same schedule after the vehicle instillation in both eyes. Two hours after carteolol instillation, iridial blood flow rate and NB_{iris} significantly increased to $127 \pm 8\%$ and $122 \pm 9\%$ (mean \pm SEM, $n = 8$) of the baseline. Unilateral instillation of carteolol significantly reduced intraocular pressure by about 9 mm Hg in both the carteolol- and vehicle-treated eyes ($P < 0.001$, analysis of variance); and NB_{iris} was significantly increased by about 20% in both eyes ($P < 0.001$, analysis of variance), compared with control eyes. **Jpn J Ophthalmol 1998;42:180-185** © 1998 Japanese Ophthalmological Society

Key Words: Carteolol, iris tissue circulation, laser speckle method, microspheres, rabbit eye.

Introduction

Beta adrenergic antagonists are commonly used as topical hypotensive agents for treatment of glaucoma. Because beta receptors, especially beta-2, mediate vascular smooth muscle relaxation, and because blockage of them generally introduces vasoconstriction in various tissues,^{1,2} topical beta blockers can affect microcirculation in the anterior uvea. In fact, topical application of timolol, the most widely used topical beta blocker with beta-1 and beta-2 blocking effects, shows vasoconstriction³ and blood flow suppression⁴ in the anterior uvea after topical application, although such conclusions have not always been confirmed by other reports.⁵⁻¹⁰ Moreover, topical application of betaxolol, another commercially available beta blocker with beta-1 selectivity and Ca²⁺ antagonistic effect,¹¹⁻¹⁵ tends to af-

fect ocular circulation favorably.¹⁶ Carteolol possesses beta-1 and beta-2 blocking effect and some accelerative properties on peripheral circulation, intrinsic sympathomimetic activity (ISA),¹⁷⁻²⁰ and the effect of releasing endothelium-dependent relaxing factor (EDRF) or prostacyclin.¹⁷ It possibly has an effect on ocular circulation that is different from timolol and betaxolol. Topical carteolol reportedly increases blood flow in some ocular tissues²¹⁻²³ but not in others.²⁴ No previous reports are available, however, about the effect of carteolol on the circulation in the anterior uvea.

Iridial circulation in a living eye can be monitored noninvasively by the laser speckle method with reasonable reliability.²⁵⁻²⁷ In the present study, we investigated the effect of a single instillation of 2% carteolol on iridial tissue circulation in pigmented rabbit eyes using this method. First, iridial blood flow before and after the instillation was determined by the microsphere technique and compared to the laser speckle results obtained simultaneously. Second, the consecutive changes of intraocular pressure (IOP) and normalized blur of the iris (NB_{iris}),²⁷ a quantita-

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tive index of iridial tissue blood velocity, was monitored for 2.5 hours after instillation.

Materials and Methods

Animal

Dutch rabbits weighing 1.5 to 2.0 kg were used and handled in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Vision and Ophthalmic Research. The animals were exposed to alternating 12-hour periods of light and dark (light on at 4:00 AM) for at least 3 weeks before use. General anesthesia was induced by 1 g/kg of urethane intravenously, and small doses of urethane were added if necessary during the experiments. Maintenance of body temperature was assisted with a heating pad, but no artificial ventilation was used.

Method of Measurement of Iridial Tissue Circulation

An apparatus²⁵⁻²⁷ used in this study included a fundus camera equipped with a diode laser (wavelength = 808 nm, laser power = 3 mW on the iris surface) and an image sensor. The iris surface, on which the laser beam was focused, was illuminated by a halogen lamp. Permeability of the infrared laser into the pigmented rabbit iris is not known, but its choroid involving the retinal pigment epithelium reportedly absorbed about 50 or 60% of the infrared radiation.²⁸ This indicates that the diode laser beam also penetrates into the iris of the pigmented rabbit. The scattered laser light was projected onto the image sensor, on which the laser speckle pattern appeared, and the normalized blur (NB), which is nearly equivalent to the reciprocal of the speckle contrast and represents tissue blood velocity,^{25,26} was calculated. Results were displayed as color graphics of the two-dimensional variation of NB levels over the measurement field. Unexpected eye movement and pupillary action, even though they should be suppressed under a parasympathetic-dominant situation by general anesthesia,²⁹ could be easily detected by the method described previously.²⁵⁻²⁷ If they happened, the measurement was discarded and another attempt was made immediately.

For measurement of the iridial blood velocity, the laser beam was focused on the temporal midsite of the iris—at 3 o'clock in the left eye and at 9 o'clock in the right eye.²⁷ The average NB over the 1.07×1.07 mm² area on the iris surface, which corresponds to 100×100 pixels on the sensor plane, was measured and stocked to obtain NB_{iris}, the average of consecutive NB measurements for half a second.²⁷ The coef-

ficients of reproducibility of NB_{iris} at 5-minute and 24-hour intervals were 9 and 14%, respectively.²⁷ The intensity of the halogen lamp illumination was held constant, and the pupillary diameter (PD) of the rabbit was measured with calipers at every NB_{iris} measurement.

Effect of Topical Carteolol

Comparison of NB_{iris} to blood flow rate determined by microsphere technique. After induction of general anesthesia, the carotid artery and the ipsilateral femoral artery of a rabbit were cannulated. Immediately after NB_{iris} measurements in the eye contralateral to arterial cannulation, nonlabeled red microspheres (15 ± 0.3 μ m, 10^7 spheres/mL; E-Z TRAC colored microspheres; E-Z TRAC, Los Angeles, CA, USA) were injected into the left ventricle and a reference blood sample was obtained from the femoral artery. Then 20 μ L of 2% carteolol (carteolol group) (Mikelan, Otsuka Pharmaceutical, Tokyo) or the vehicle (control group) was instilled into an eye contralateral to arterial cannulation. Two hours after instillation, NB_{iris} measurements and microsphere injection were repeated with blue microspheres. The IOP and PD in the treated eye were also measured using a calibrated applanation pneumotonometer (Alcon, Fort Worth, TX, USA) and calipers. Systemic parameters were monitored before and 2 hours after instillation. Animals were then sacrificed by an intravenous overdose of sodium pentobarbital, and the eyes treated with carteolol were enucleated and the iridial blood flow rates before and 2 hours after instillation were determined.²⁷

Consecutive changes of NB_{iris} and IOP after topical instillation of carteolol. After induction of general anesthesia, one randomly chosen eye of each animal received 20 μ L of 2% carteolol, and the fellow eye received the vehicle at 6:00 PM (carteolol group). To provide a control, both eyes received the vehicle at 6:00 PM in the other groups of rabbits treated similarly to the carteolol group (control group). During the experiment, NB_{iris}, IOP, and PD in both eyes of the carteolol group and one randomly chosen eye of the control group were measured before and 0.5, 1, 1.5, 2, and 2.5 hours after instillation. Intraocular pressure and PD were measured simultaneously with a calibrated applanation pneumotonometer and calipers.

Results

Comparison of NB_{iris} to Blood Flow Rate as Determined by the Microsphere Technique

We examined 16 rabbits (8 from the carteolol group, 6 from the control group), prepared for the

Table 1. Values of Systemic Condition Parameters at the Time of Microsphere Injection

	Carteolol Treated		Control	
	Before ^a	After 2 Hours ^b	Before ^a	After 2 Hours ^b
FABP (mm Hg)	116.0 ± 7.9	102.5 ± 4.2	122.4 ± 3.5	113.9 ± 8.9
Pulse rate (/min)	315 ± 12	250 ± 8	322 ± 14	331 ± 9
BT (°C)	38.5 ± 0.2	37.8 ± 0.2	37.9 ± 0.2	37.7 ± 0.3
pH	7.43 ± 0.01	7.48 ± 0.01	7.41 ± 0.04	7.40 ± 0.03
PCO ₂ (mm Hg)	29.5 ± 1.4	31.1 ± 1.7	31.5 ± 3.5	31.6 ± 3.7
Po ₂ (mm Hg)	98.2 ± 3.4	101.5 ± 2.4	102.2 ± 2.6	106.7 ± 3.7

Values are mean ± SEM (n = 8). FABP: Femoral arterial blood pressure. BT: Body temperature.

^aEyes before instillation.

^bEyes 2 hours after instillation.

microsphere experiment, whose systemic parameters before instillation were in the normal range^{30,31} (Table 1). There were no significant differences in baseline NB_{iris}, IOP, and PD in both groups. Although pulse rate significantly decreased 2 hours after carteolol instillation ($P = 0.0022$, paired t -test), no systemic parameters dissociated far from the normal range.^{30,31} Pupillary diameter showed no significant change during the experimental period. Intraocular pressure decreased 2 hours after instillation from 30.1 ± 2.2 mm Hg (mean ± SEM) to 25.8 ± 2.3 mm Hg ($P = 0.0202$, paired t -test) in the carteolol group but did not change significantly in the control group. The NB_{iris} and the blood flow measured by the microsphere technique are shown in Table 2, with the ratio to the baseline. Both the blood flow rate and the NB_{iris} in the carteolol group increased significantly 2 hours after treatment ($P = 0.0332$ and 0.0401 , respectively; paired t -test), and the ratio averaged 1.27 for the former and 1.22 for the latter.

Consecutive Changes of NB_{iris} and IOP After Topical Instillation of Carteolol

There were no significant differences in baseline NB_{iris}, IOP, and PD in both groups, and no significant change in PD was seen during the experiment.

Consecutive changes of IOP are shown in Figure 1. The changes in both eyes in the carteolol group were significantly different from the control eyes ($P = 0.0000007$ and 0.0000006 , respectively; analysis of variance), with a marked decrease 1, 1.5, 2, and 2.5 hours after instillation in the carteolol-treated eyes ($P = 0.0077$, 0.0122 , 0.0037 , and 0.0038 , respectively; unpaired t -test with Bonferroni's correction for multiple comparison) and 1 and 2.5 hours in the fellow eye ($P = 0.0009$ and 0.0153 , respectively; unpaired t -test with Bonferroni's correction for multiple comparison). There was no significant difference between both eyes of the carteolol group.

Changes of NB_{iris} are shown in Figure 2. The changes in both eyes in the carteolol group were significantly different from control eyes ($P = 0.000005$ and 0.000005 , respectively; analysis of variance), with a marked increase 2.5 hours after instillation in both eyes ($P = 0.0098$ and 0.0096 , respectively; unpaired t -test with Bonferroni's correction for multiple comparison). There was no significant difference in NB_{iris} between both eyes of the carteolol group.

Discussion

In this study, we used urethane anesthesia, which has relatively little effect on peripheral circulation.³²

Table 2. Iridial Blood Flow Rate and NB_{iris} Before and After Topical Instillation of Carteolol

	Before ^a	After 2 Hours ^b	Ratio to Baseline ^c
Carteolol treated (n = 8)			
Blood flow rate (mg/min/mg tissue)	0.95 ± 0.17	1.16 ± 0.20	1.27 ± 0.08
NB _{iris} ^d	10.5 ± 0.5	12.7 ± 0.7	1.22 ± 0.09
Control (n = 6)			
Blood flow rate (mg/min/mg tissue)	0.98 ± 0.14	1.01 ± 0.10	1.08 ± 0.10
NB _{iris}	10.5 ± 0.7	11.0 ± 1.22	1.04 ± 0.07

Values are mean ± SEM.

^aEyes before instillation of 2% carteolol.

^bEyes 2 hours after instillation of 2% carteolol.

^cThe ratio 2 hours after instillation to the baseline.

^dNormalized blur of the iris.

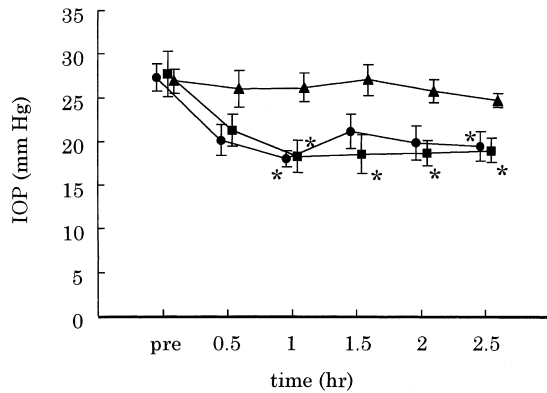


Figure 1. Changes of intraocular pressure (IOP) after instillation in the carteoled-treated eyes (■) and the vehicle-treated eyes (●) in the carteoled group as well as randomly selected eyes in the control group (▲). Each plot represents mean IOP with SEM (error bars) in eight rabbits. * $P < 0.05$ by unpaired t -test with Bonferroni's correction for multiple comparison for difference as compared to the control group.

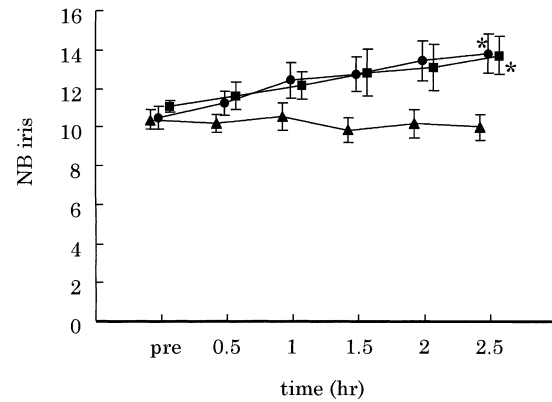


Figure 2. Changes of normalized blur of the iris (NB_{iris}) after instillation in the carteoled-treated eyes (■) and the vehicle-treated eyes (●) in the carteoled group as well as randomly selected eyes in the control group (▲). Each plot represents mean NB_{iris} with SEM (error bars) in eight rabbits. * $P < 0.05$ by unpaired t -test with Bonferroni's correction for multiple comparison for difference as compared to the control group.

However, all commonly used general anesthetics, including urethane, affect results in both physiologic and pharmacologic studies.^{32,33} Therefore, we compared current results with those from similarly prepared control rabbits. Although pulse rate significantly decreased after carteoled instillation in the microsphere experiment, probably reflecting a cardiac beta-blocking effect of topical carteoled, the other parameters did not show significant changes during the experiment.

The iridial blood flow rate before instillation of carteoled, as found by the colored microsphere technique in this study, coincides with the rate determined by the radioactive microspheres in a normal rabbit eye.^{34,35} The results of two separate methods—the microsphere technique and the laser speckle method—were very similar regarding the increasing ratio 2 hours after carteoled instillation. These findings substantiate the notion that NB_{iris} , primarily a quantitative index of tissue blood velocity, can also apply to studies of changes in iridial blood flow rate. In the other experiment, NB_{iris} gradually increased after a single instillation of carteoled in both carteoled- and vehicle-treated eyes as compared with control eyes, which indicates that topical carteoled significantly increases not only the iridial tissue blood velocity but also the iridial blood flow rate.

Between the two experiments, a discrepancy was found regarding IOP reduction after carteoled instillation. This may result from the differing depths of general anesthesia, because deeper anesthesia was necessary for the microsphere experiment than in the time-course experiment.

Topical carteoled is a commonly used ocular hypotensive agent that possesses beta-1 and beta-2 adrenergic blocking action. The beta-2-blocking effect generally causes vasoconstriction in peripheral tissues.^{1,2} Timolol, which is the most widely used topical nonselective beta blocker, has been studied extensively regarding its effect on blood flow in various ocular tissues including the iris and the ciliary body. Although timolol's effects on iridial circulation have not been verified by previous reports, Van Buskirk et al³ reported constriction of ciliary body arteries after long-term topical instillation, and Watanabe and Chiou⁴ reported reduction of blood flow rate as measured with microspheres after a single instillation of 0.25% timolol. These results suggest that timolol has the ability to reduce the iridial tissue blood flow through its beta-2 antagonistic effect. Therefore, the present results cannot be explained simply by the beta-blocking effect of carteoled.

We previously reported that NB_{iris} decreased with acute reduction of ocular perfusion pressure.²⁷ During consecutive NB_{iris} measurements after topical carteoled, the maximum reduction of IOP was approximately 9 mm Hg in both the carteoled- and vehicle-treated eyes. Although blood pressure of the rabbits was not monitored in this experiment, the reduction of blood pressure from 116 mm Hg to 103 mm Hg was seen in the microsphere experiment. Because ocular perfusion pressure is calculated by the subtraction of IOP from blood pressure,³⁶ and some reduction in blood pressure was expected, IOP reduction should not be a main factor in the increasing

effect of carteolol on iridial circulation as seen in the current study.

In addition to its beta-blocking effect, carteolol has intrinsic sympathomimetic activity (ISA) and the releasing effect of EDRF or prostacyclin, both of which reportedly increase peripheral circulation.¹⁷⁻²⁰ Intrinsic sympathomimetic activity of carteolol is seen at a concentration of 3 to 10 times higher than that of the beta-blocking effect, which is about 10^{-7} M,^{17,19} and carteolol shows the EDRF or prostacyclin releasing effect at a concentration of 10^{-8} M or higher in isolated canine coronary arteries.¹⁷ According to Fujio and Kitazawa,³⁷ the concentration of carteolol, 1 hour after a single instillation, was equivalent to 5.5×10^{-6} M and 1.5×10^{-6} M in the anterior chamber and the iris, respectively, of the treated eyes of pigmented rabbits. The concentration in the eye contralateral to drug instillation, which was only available for the albino rabbits, was 10 times lower than the concentration in the carteolol-instilled eye.³⁸ Therefore, carteolol concentration in the iris not only of the treated eye but also of the contralateral eye should possibly reach a level that is sufficient to increase iridial blood flow through its ISA, EDRF, or prostacyclin releasing effect.

The pharmacokinetics of topical carteolol in human eyes is not well known. The concentration of timolol and betaxolol, whose pharmacokinetics after topical instillation are not thought to dissociate far from carteolol, is 3.8×10^{-6} M and 11.9×10^{-6} M, respectively, in the human anterior chamber several hours after topical instillation,³⁹ and a similar concentration level is shown in rabbits (5.5×10^{-6} M³⁹ and 5.8×10^{-6} M,¹¹ respectively). Therefore, carteolol concentration after topical instillation is supposed to be sufficient to cause ISA and the EDRF or prostacyclin releasing effect in the human anterior chamber.

Intraocular pressure reduction after topical beta blockers is caused mainly by the suppression of the aqueous humor.^{40,41} Some investigators have supposed that reduction of blood flow in the anterior uvea, after topical timolol, relates to the decrease of aqueous production.⁴ The current results, however, suggest that the suppressive effect of beta blockers on the aqueous humor is independent of their own efficacy on iridial circulation, because carteolol also reduces aqueous production after topical application.⁴⁰ Although clinical benefits of increasing iridial circulation after topical agents are still unclear, carteolol may have favorable properties that promote avoidance of chronic ischemia in the ocular tissue, which is feared in relation to use of topical beta blockers for treatment of glaucoma.

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