Effects of Topical Latanoprost on Optic Nerve Head Circulation in Rabbits, Monkeys, and Humans

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Purpose. To evaluate the effect of topically administrated latanoprost on optic nerve head (ONH) circulation in Dutch rabbits, cynomolgus monkeys, and normal humans.

Methods. The ONH tissue blood velocity (NB_{ONH}) was determined using the laser speckle method. Latanoprost (0.005%, 30 μl) was instilled into one eye, and vehicle into the other eye as a control. In rabbits, NB_{ONH} was measured for 90 minutes after a single instillation and before and after a 7-day once-daily instillation regimen. In monkeys, NB_{ONH} was measured before and after 1, 4, and 7 days of a once-daily instillation regimen. The effect of intravenous indomethacin on the latanoprost-induced NB_{ONH} change was also studied in rabbits and monkeys. In humans, the time-course changes in NB_{ONH} were measured for 4.5 hours before and after a 7-day once-daily instillation regimen. Intraocular pressure (IOP) and systemic parameters were simultaneously studied in each experiment. All measurements were performed by investigators masked to the experimental condition.

Results. Latanoprost significantly increased NB_{ONH} 10% to 19% in treated eyes after a single instillation ($P = 0.035$) or 7-day instillation regimen ($P = 0.035$) in rabbits, after a 4-day ($P = 0.035$) or 7-day ($P = 0.035$) instillation regimen in monkeys, and after a 7-day ($P = 0.013$) instillation regimen in humans, whereas there were no significant changes in the vehicle-treated eyes in any of the experiments ($P > 0.5$). Pretreatment with indomethacin (5 mg/kg) abolished the NB_{ONH} increase but not the IOP reduction in latanoprost-treated eyes in rabbits and monkeys. IOP remained unchanged in both eyes in rabbits ($P > 0.4$), whereas it significantly decreased only in latanoprost-treated eyes in monkeys ($P < 0.05$) and humans ($P < 0.05$).

Conclusions. Topical latanoprost significantly increased ONH blood velocity only in treated eyes in rabbits, monkeys, and humans. This effect was independent of the IOP-reducing effect of latanoprost and probably was associated with local penetration of the drug and the production of endogenous prostaglandins. (*Invest Ophthalmol Vis Sci.* 2001;42:2957–2963)

Latanoprost, a recently developed prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)–related FP receptor agonist compound,[1] is one of the most potent ocular hypotensive eye drops in patients with glaucoma.[2–8] Although intraocular pressure (IOP) is consistently the major risk factor for glaucoma, recent studies indicate that impaired circulation in the optic nerve head (ONH) has a crucial role in glaucomatous optic neuropathy.[9–11]

Previous studies have suggested that topically instilled timolol or betaxolol over a long period can accumulate in the periocular tissues and reach the retrobulbar space in sufficient concentration to have pharmacologic effects on the retrobulbar vessels.[12–14] $PGF_{2\alpha}$ has vasoconstricting or vasodilating effects, depending on its concentration, the nature of the vascular bed, or the animal species.[15–18] Latanoprost also has vasoconstricting effects at higher concentrations[19] or increases blood flow in the anterior sclera after topical instillation.[20]

Grunwald[21–23] and Gupta et al.[24] reported significant correlations between changes in blood velocity in the retinal vein and those in ocular perfusion pressure (OPP) caused by IOP reduction after treatment with topical timolol or betaxolol. Latanoprost has potent IOP-lowering and OPP-increasing effects,[2–8] and, in fact, McKibbin and Menage[8] reported that topical latanoprost increased pulsatile ocular blood flow in patients with normal-tension glaucoma by increasing OPP. If latanoprost, one of the principal and most potent antiglaucoma eye drops presently available, affects the circulation in the ONH, the primary site of damage in glaucoma, it would be of clinical interest, independent of the mechanisms attributed to its OPP-increasing effects or direct effects on the retrobulbar vessels. There are limited data available on the in vivo effects of latanoprost on ONH circulation. Seong et al.[25] reported that a single instillation of latanoprost did not significantly change the ONH or peripapillary retinal blood flow measured using Heidelberg retinal flowmetry (HRF; Heidelberg Engineering, Heidelberg, Germany) in normal humans.

In the present study, we examined the effects of not only a single instillation but also a 7-day once-daily instillation regimen of latanoprost on ONH circulation in rabbits, monkeys, and normal humans, by using the noninvasive laser speckle method.[26,27] Rabbits were used in the present study, because latanoprost has no apparent IOP-lowering effects in this species.

Materials and Methods

Instruments

ONH circulation was evaluated using the laser speckle method.[26,27] An apparatus consisting of a fundus camera was equipped with a diode laser (wavelength: 808 nm), with the laser beam focused on the fundus and scattered laser light detected with an image sensor on which a speckle pattern appeared. The difference between the average of the speckle intensity (I_{mean}) and the speckle intensity for successive scans 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). NB is essentially equivalent to the reciprocal of speckle contrast described by Fercher and Briers[28,29] and is thought to be an indicator 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 NB of the largest rectangular field free of visible

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surface vessels on the ONH was expressed as NB_{av}. The measurement field was located on the lower quadrant of the ONH in rabbits and on the temporal quadrant of the ONH in monkeys and humans. The measurement size was approximately 0.40 × 0.40 mm in rabbits and monkeys and ranged from 0.22 × 0.29 mm to 0.40 × 0.54 mm in humans. The size varied with the individual human subject because of the necessity to avoid surface vessels. In rabbit and monkey experiments, NB_{av} was measured for 2 seconds, during which no apparent eye movements were observed, five times at 30-second intervals, and the average of three results, excluding the maximum and the minimum values, was adopted as the NB_{ONH}. In human experiments, NB_{av} was measured for 5 seconds, during which no eye apparent movements were observed, and the average over three heartbeats was adopted as NB_{ONH}.

The IOP was measured using a calibrated applanation pneumotonometer (Alcon Laboratories, Fort Worth, TX) in rabbits and monkeys and a Goldmann applanation tonometer in humans after instillation of topical anesthetic (0.4% oxybuprocaine hydrochloride, Benoxil; Senju, Osaka, Japan). In rabbits, the blood pressure and pulse rate were measured in the foreleg with an automatic animal sphygmomanometer (BP-9000; Softron, Tokyo, Japan) and in monkeys in the forearm with a infant sphygmomanometer (SP-8800; Nihonkoden, Tokyo, Japan). 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. In monkeys, arterial O_2 saturation (SaO_2), and body temperature were monitored using a pulse oxygen meter (OLV-1200; Nihonkoden) and thermometer (Thermopit IT-500M; Nipro, Osaka, Japan).

All measurements, including NB_{ONH}, IOP, and systemic parameters were performed by investigators who were blind to the drug treatments. All NB_{ONH} measurements were stored on magneto-optical disks as color graphics and NB_{ONH} was later determined by an investigator blind to the drug treatments.

Drugs

Latanoprost (13,14-dayihydro-17-phenyl-18,19,20-trinor-$PGF_{2\alpha}$-isopropyl-ester) 0.005% ophthalmic solution (Xalatan; Pharmacia, Uppsala, Sweden) was used and the vehicle solution was made in the laboratory according to the published data[4] and sterilized through filters (0.2 μm in pore size; Japan Millipore, Tokyo, Japan).

Rabbit Experiment

Dutch rabbits (ages, 10–13 months; weight, 1.5–2.5 kg; sex, irrespective) were used and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animals were entrained to a light schedule of alternating 12-hour periods of light and dark (lights on at 4 AM) for at least 3 weeks before use.

Single Instillation

General anesthesia was induced by 1 g/kg of intravenous urethane at 10 AM. Body temperature was maintained using a heating pad, but no artificial ventilation was used. Approximately 30 minutes after induction of general anesthesia, the pupils were dilated with one drop 0.4% tropicamide in each eye. Fifteen minutes thereafter, NB_{ONH} and IOP in both eyes, blood pressure, and pulse rate were measured, as described earlier. A color fundus photograph (Polaroid; Cambridge, MA) was taken to record the site of the NB_{ONH} measurement. Latanoprost (30 μl) was instilled into one randomly chosen eye and vehicle into the other eye. NB_{ONH} and IOP in both eyes, blood pressure, and pulse rates were measured 30, 60, and 90 minutes after instillation (normal group). The same experiment was performed in other groups of rabbits pretreated with intravenous injection of indomethacin at a dose of 5 mg/kg (indomethacin group) or pretreated with the same volume of the indomethacin solvent (indomethacin-solvent group) 15 minutes before instillation of latanoprost or vehicle. To study the effects of latanoprost on aqueous barrier permeability, 0.05 mg/kg of 10% fluorescein sodium (Fluorescite; Alcon Laboratories) was intravenously injected 10 minutes after instillation of latanoprost or vehicle in a separate group of rabbits similarly treated (fluorescein group). Forty minutes after instillation, the dye concentration in the anterior chambers of both eyes was measured fluorophotometrically.[30]

Seven-Day Instillation

On the first experimental day, after general anesthesia and pupil dilation, the NB_{ONH} and IOP in both eyes, blood pressure, and pulse rates were measured at 10:45 AM. After these measurements, 30 μl latanoprost was instilled into one randomly chosen eye and vehicle into the other eye at 11 AM for 7 days. On the seventh experimental day, 15 minutes before the final instillation, the NB_{ONH}, IOP, blood pressure, and pulse rate were measured after general anesthesia. This was followed by instillation at 11 AM, and the same measurements were made 30 minutes later (11:30 AM). Thereafter, indomethacin was intravenously administrated at a dose of 5 mg/kg at 11:45 AM, and the same measurements were repeated 30, 60, and 90 minutes after the indomethacin injection.

Monkey Experiments

Eight adult cynomolgus monkeys (ages, 5–8 years; weight, 3–7 kg; sex, two males and six females) were used and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All examinations were performed with the monkey sitting in a monkey chair.[31]

On the first experimental day, after general anesthesia was induced by ketamine hydrochloride (Ketalar; Sankyo, Tokyo, Japan) at a dose of 8 to10 mg/kg intramuscularly, and pupil dilation was induced with one drop of tropicamide in both eyes. The NB_{ONH}, IOP, blood pressure, pulse rate, SaO_2, and body temperature were measured at 12 PM. Starting on the second experimental day, 30 μl latanoprost was instilled once daily into one randomly chosen eye and vehicle into the other eye at 8 AM for 7 days. At 12 PM on the second, fifth, and eighth experimental days, the same measurements were repeated after general anesthesia and bilateral pupil dilation (normal group).

The influence of indomethacin on the latanoprost-induced effects was studied using five of the same eight monkeys after a 4-week washout period (indomethacin group). The same protocol was followed, except that measurements on the second and fifth experimental days were omitted, and 5 mg/kg indomethacin was intravenously injected at 11:30 AM on the eighth experimental day.

Human Experiments

Eleven healthy male volunteers (ages, 22–39 years), with or without mild refractive errors but without any history of systemic or ocular diseases, were included. All subjects had best corrected visual acuities of 20/20 or better, an IOP of 19 mm Hg or less, and normal anterior segments and fundi. This study was approved by the Institutional Ethics Committee of the University of Tokyo and adhered to the tenets of the Declaration of Helsinki. A written consent form was signed by each subject before participation in the study.

On the first experimental day, after both pupils were dilated with one drop of tropicamide, the NB_{ONH} and IOP in both eyes, blood pressure, and pulse rate were measured with subjects in the sitting position at 9 AM. The vehicle solution (30 μl) was instilled immediately in both eyes. The same measurements were repeated 90, 180, and 270 minutes after the instillation. From the second to eighth experimental days, a drop of latanoprost was instilled in one randomly chosen eye and vehicle in the other eye once a day at 9 AM in a double-blind manner. The same measurement protocol as on the first experimental day was repeated on the eighth day. On the days of measurements, the subjects were instructed to strictly refrain from smoking and drinking beverages containing caffeine for at least 8 hours before and during the experiment.

Data Analysis

Because the laser speckle method dose not give an absolute value for the tissue blood velocity, the NB_{ONH} is not suited for direct interindi-

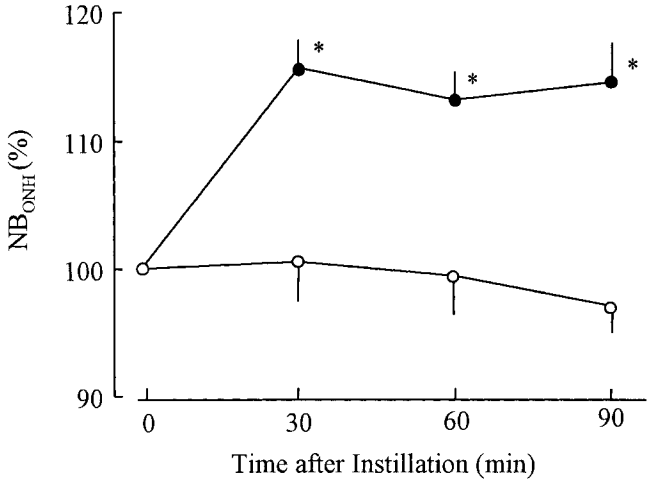

FIGURE 1. Time course of changes in NB_{ONH} after a single instillation of latanoprost (●) or vehicle (○) in eyes of a normal group of Dutch rabbits ($n = 8$). Each *plot* represents the ratio of NB_{ONH} to baseline, with a *bar* denoting SE. $^*P < 0.05$ by Wilcoxon signed rank test with Bonferroni correction for the bilateral difference.

vidual comparison.[26,27] However, this method yields good reproducibility in the same tissue of an individual, and its change relative to baseline correlates well with the relative change of blood flow determined using the microsphere method[26,27] or hydrogen gas clearance method.[32,33]

Therefore, the time change in the NB_{ONH} after the drug or vehicle solution instillation was compared between the latanoprost and vehicle-treated eyes based on the value normalized to baseline (ratio). The Wilcoxon signed rank test was applied to examine the difference from baseline or differences in the ratio between both eyes. In analyses including multiple comparisons, the probabilities were divided according to the Bonferroni method, and $P < 0.05$ was thereafter considered to be statistically significant.

RESULTS

Rabbit Experiments

A preliminary experiment revealed that coefficients of reproducibility of NB_{ONH} measurements at 30-, 60-, and 90-minute and 7-day intervals in rabbits were 9.2% ± 1.3% (mean ± SE, $n = 16$), 11.9% ± 2.7%, 11.6% ± 2.3%, and 12.9% ± 1.7%, respectively, and those of IOP measurements were 8.7% ± 2.4%, 4.8% ± 2.0%, 6.2% ± 1.4%, and 5.3% ± 2.0%.

Single Instillation

There were no significant changes during the experimental period in any of the form groups, and IOP and OPP in both eyes, blood pressure, and pulse rate were within the normal range for healthy rabbits.[34]

In the normal group ($n = 8$, Fig. 1), there was no significant difference between the NB_{ONH} in the latanoprost-treated eyes and that in the vehicle-treated eyes before instillation, averaging 11.91 ± 1.42 and 13.11 ± 1.51, respectively ($P > 0.4$). In the latanoprost-treated eyes, NB_{ONH} significantly increased by 13% to 16% from baseline at 30, 60, and 90 minutes after a single instillation ($P = 0.035$). In the vehicle-treated eyes, the NB_{ONH} did not significantly change. Bilateral differences in the ratio were also significant at 30, 60, and 90 minutes ($P = 0.035$).

In the indomethacin group ($n = 8$), no significant changes in the NB_{ONH} were seen in either latanoprost- or vehicle-treated eyes. In the indomethacin-solvent group ($n = 8$), the results were very similar to those in the normal group: In the latanoprost-treated eyes, the NB_{ONH} increased significantly by approximately 15% at 30, 60, and 90 minutes ($P = 0.035$), whereas the NB_{ONH} in the vehicle-treated eyes remained almost unchanged.

In the fluorescein group ($n = 8$), fluorescein concentration in the anterior chamber was $1.78 \pm 0.37 \times 10^{-7}$ g/ml in the latanoprost-treated eyes and $1.70 \pm 0.34 \times 10^{-7}$ g/ml in the vehicle-treated eyes; there was no significant bilateral difference ($P > 0.5$).

Seven-Day Instillation

There were no significant changes from baseline in IOP or OPP in either eye, blood pressure, or pulse rate at any time point, and values were within the normal range for healthy rabbits.[34]

The NB_{ONH} in the latanoprost-treated eyes at 10:45 and 11:30 AM on the seventh experimental day significantly increased from baseline at 10:45 AM on the first experimental day by 26% and 25%, respectively ($P = 0.025$, 0.025, $n = 10$). Bilateral differences in the ratio were also significant at 10:45 and 11:30 AM on the seventh experimental day ($P = 0.025$, 0.025, Fig. 2). There were no significant differences from

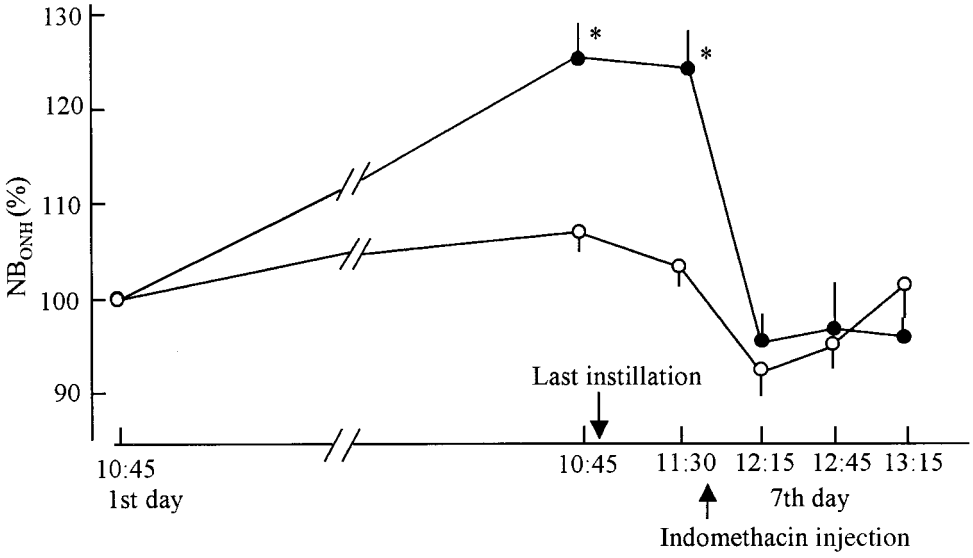

FIGURE 2. Time course of changes in NB_{ONH} after 7-day instillation of latanoprost (●) or vehicle (○) in eyes of Dutch rabbits treated with indomethacin at 11:30 AM on the seventh experimental day ($n = 10$). Each *plot* represents the average of the ratio of NB_{ONH} to baseline at 10:45 AM on the first day, with a *bar* denoting SE. $^*P < 0.05$ by Wilcoxon signed rank test with Bonferroni correction for the bilateral difference.

TABLE 1. Systemic Parameters of the Monkeys before and after the 7-day Instillation of Latanoprost

	Experimental Day			
	Baseline	**2**	**5**	**8**
BP_m (mm Hg)	70.6 ± 7.4	65.4 ± 7.1	67.9 ± 7.9	71.5 ± 7.5
Pulse rate (beats/min)	159.9 ± 12.1	164.5 ± 9.9	171.8 ± 8.3	153.8 ± 7.4
BT(°C)	36.6 ± 0.4	36.4 ± 0.5	36.5 ± 0.4	36.5 ± 0.5
SaO_2	99.1 ± 0.4	98.7 ± 0.7	98.4 ± 0.8	98.9 ± 0.7

Data are mean ± SEM (n = 8). BP_m, mean arterial blood pressure; BT, body temperature; SaO_2, saturation of arterial O_2.

baseline or bilateral differences in the ratio were seen after the injection of indomethacin ($P > 0.4$).

Monkey Experiments

A preliminary experiment indicated that the coefficient of reproducibility of NB_{ONH} and IOP at the 1-week interval was 7.3% ± 1.3% and 5.6% ± 2.1%, respectively (n = 8).

Blood pressure, pulse rate, body temperature, and SaO_2 when the NB_{ONH} was measured under general anesthesia in the normal group are shown in Table 1. There were no significant changes in these parameters during the experimental period. IOP and OPP are shown in Figure 3. OPP was calculated according to the formula: $OPP = 2/3BP_m - IOP$.[35] In the latanoprost-treated eyes on the fifth and eighth experimental days, IOP was significantly lower (paired t-test, P = 0.008, 0.003), whereas OPP was significantly higher (P = 0.039, 0.003), compared with the vehicle-treated eyes (Fig. 3). There was no significant difference between the NB_{ONH} in the latanoprost-treated eyes and that in the vehicle-treated eyes before instillation, averaging 9.04 ± 0.55 and 9.24 ± 0.81, respectively ($P > 0.5$). NB_{ONH} significantly increased from baseline on the eighth day by 19.0% (P = 0.035) and tended to increase on the fifth day (P = 0.053) in the latanoprost-treated eyes, whereas there was no significant change in the vehicle-treated eyes. There was also a significant difference in the ratio on the fifth and eighth experimental days (P = 0.035, 0.035, Fig. 4).

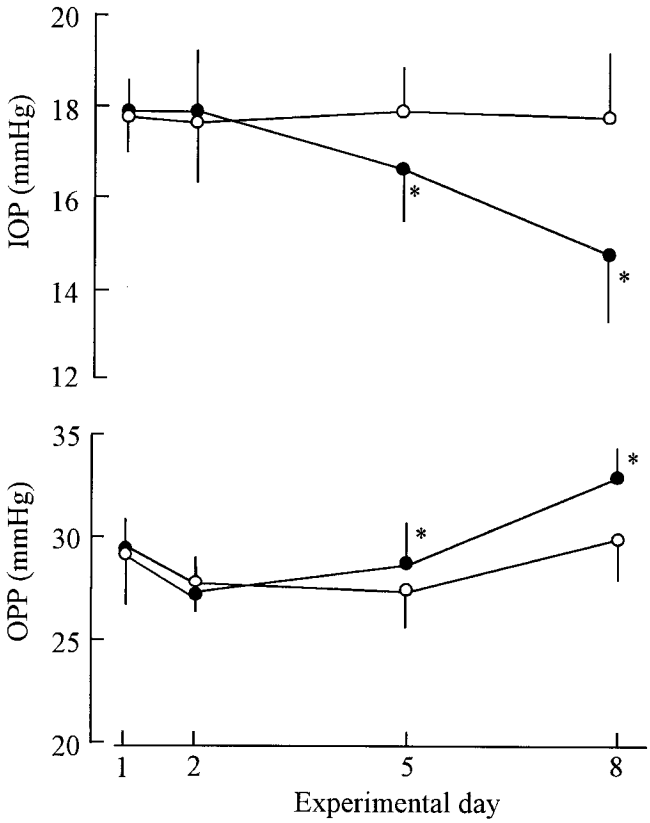

FIGURE 3. Changes in IOP (*top*) or OPP (*bottom*) in eyes treated with latanoprost (●) or vehicle (circo]) in monkeys (n = 8). Each *plot* represents the average IOP or OPP, with a *bar* denoting SE. *$P < 0.05$ by paired t-test with Bonferroni correction for the bilateral difference.

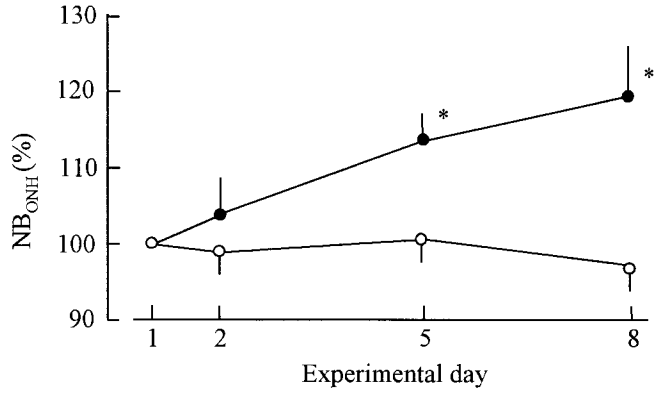

FIGURE 4. Changes in NB_{ONH} in eyes treated with latanoprost (●) or vehicle (○) in monkeys (n = 8). Each *plot* represents the ratio of NB_{ONH} to baseline, with a *bar* denoting SE. *$P < 0.05$ by Wilcoxon signed rank test with Bonferroni correction for the bilateral difference.

In five indomethacin-treated monkeys, IOP was significantly lower in the latanoprost- than that in the vehicle-treated eyes on the seventh (8 AM) and eighth experimental (12 PM) days (P = 0.046, 0.004, Fig. 5). There were no significant changes from baseline, however, in NB_{ONH} in the latanoprost- or vehicle-treated eyes.

Human Experiments

The systemic parameters of the subjects during the experiment are shown in Table 2. Blood pressure and pulse rate did not change significantly during the experiment.

There was no bilateral difference in IOP or OPP throughout the experiment on the first experimental day. On the eighth experimental day, IOP was significantly lower in the latanoprost-treated eyes than in the vehicle-treated eyes at all mea-

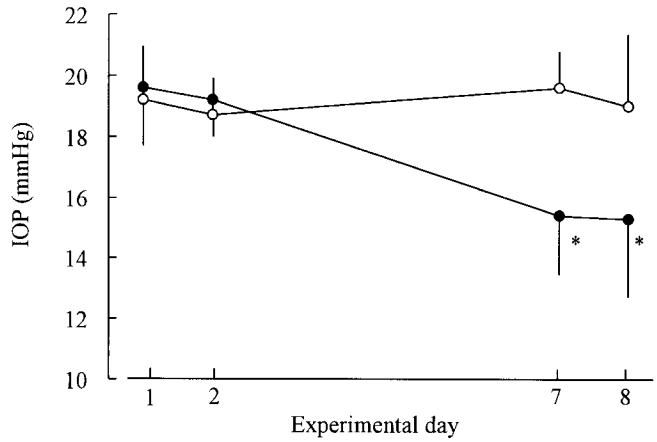

FIGURE 5. Changes in IOP in eyes treated with latanoprost (●) or vehicle (○) in indomethacin-treated monkeys (n = 5). Each *plot* represents the average IOP, with a *bar* denoting SE. *$P < 0.05$ by paired t-test with Bonferroni correction for the bilateral difference.

TABLE 2. Systemic Parameters of the Human Subjects before and after the 7-day Instillation of Latanoprost

	0 Minutes	90 Minutes	180 Minutes	270 Minutes
BP_m* (mm Hg)	83.5 ± 2.1	79.8 ± 2.3	81.8 ± 2.2	81.5 ± 2.1
BP_m† (mm Hg)	77.4 ± 1.3	78.8 ± 2.1	78.5 ± 1.9	77.5 ± 2.2
Pulse rate* (beats/min)	73.0 ± 2.8	72.4 ± 1.9	71.3 ± 2.5	68.7 ± 3.0
Pulse rate† (beats/min)	71.2 ± 1.9	66.5 ± 2.4	76.0 ± 2.2	72.3 ± 2.6

Data are mean ± SEM (n = 11). BP_m, mean arterial blood pressure; * first experimental day; † eighth experimental day.

surement points ($P < 0.011$, Fig. 6,, left), whereas OPP was significantly higher ($P < 0.011$, Fig. 6, right).

On the first experimental day, there was no significant difference in the NB_{ONH} between the latanoprost- and vehicle-treated eyes, averaging 18.2 ± 2.58 and 20.12 ± 2.11 (n = 11) at 9 AM before instillation. NB_{ONH} did not change significantly during the first day of the experiment in either eye. Before instillation of latanoprost on the eighth experimental day, the NB_{ONH} did not change significantly from that obtained before instillation on the first experimental day, but 180 minutes after instillation, NB_{ONH} significantly increased by 26% (P = 0.031) and tended to increase at 90 and 270 minutes (P = 0.065, 0.051), in the latanoprost-treated eyes. In the vehicle-treated eyes, there were no significant differences on the eighth experimental day from that on the first day during the experiment period from 9 AM to 12 PM. The bilateral difference in the ratio was also significant at 90, 180, and 270 minutes (P = 0.013, 0.013 and 0.013; Fig. 7).

DISCUSSION

According to Koelle et al.,[36] the penetration depth of the near-irradiation laser (wavelength, 811 nm) in the cat optic nerve exceeds 1 mm. With the apparatus presently used, the effective depth of sampling in the ONH tissue is more than 1 mm. Using the same type of apparatus, NB_{ONH} and blood flow rate determined using the hydrogen gas clearance method (in which a hydrogen electrode is inserted into the ONH tissue to

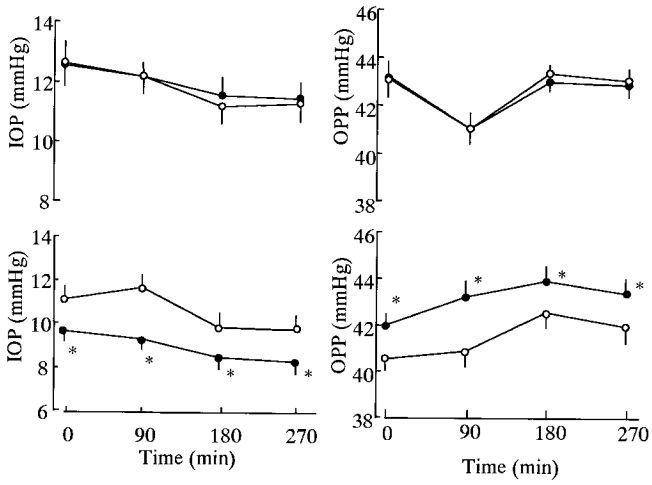

FIGURE 6. Changes in IOP (*right*) and OPP (*left*) on the first (*top*) and the eighth (*bottom*) experimental days in eyes treated with latanoprost (●) or vehicle (○) in humans (n = 8). Each *plot* represents the average IOP or OPP, with a *bar* denoting SE. *$P < 0.05$ by paired t-test with Bonferroni correction for the bilateral difference.

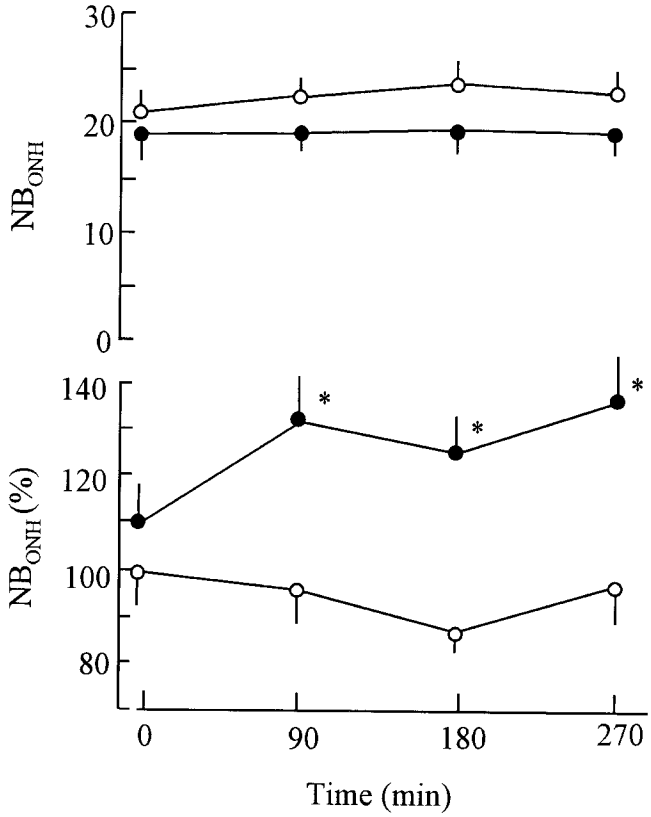

FIGURE 7. Changes in NB_{ONH} on the first day (*top*) and changes in the ratio of NB_{ONH} on the eighth day compared with the same time on the first day in the same eye (*bottom*), in eyes treated with latanoprost (●) or vehicle (○) on the first (*top*) and eighth (*bottom*) experimental days in humans (n = 11). Each *plot* represents the average of NB_{ONH} or the ratio, with a *bar* denoting SE. *$P < 0.05$ by Wilcoxon signed rank test with Bonferroni correction for the bilateral difference.

a depth of approximately 0.7 mm) were compared in rabbit eyes under various conditions.[32,33] There was a good and significant correlation between the relative change in NB_{ONH} and blood flow rate, determined using the hydrogen gas clearance method.[32,33] These results suggest that NB_{ONH}, primarily a quantitative index of blood velocity in the ONH, is also correlated with the ONH blood flow rate, at least under some conditions. Poiseuille's law can be applied not only to the large vessels but also to arterioles.[37,38] According to the Poiseuille formula,[39] blood flow in the arteriole is calculated by

$$F = \pi/128 \times \Delta P \times 1/\eta \times D^4 \quad (1)$$

where ΔP is the pressure difference along the vessel, η is the blood viscosity, and D is the vessel (blood column) diameter. Because the mean blood velocity (V_{mean}), is obtained by $F/(\eta D^2/4)$, V_{mean} can be calculated by

$$V_{mean} = \frac{1}{32} \times \Delta P \times \eta \times D^1 \quad (2)$$

Therefore, as OPP or vessel diameter increases, blood velocity increases.

In the rabbit experiment, latanoprost did not change the IOP or blood–aqueous barrier permeability significantly. These results are consistent with previous results.[40,41] A single instillation of latanoprost, however, increased the NB_{ONH}. Therefore, the increase in the NB_{ONH} observed after latanoprost instillation is probably not due to an elevation in the OPP

(reduction in the IOP) or intraocular inflammatory responses, but rather to a local vasodilatory effect of latanoprost. On the eighth experimental day, the NB_{ONH} increased not only after instillation, but also before instillation (24 hours after the last instillation). Moreover, an increase in NB_{ONH} in single or repeated instillations was suppressed by an intravenous injection of indomethacin. These findings suggest that the effect of latanoprost on ONH circulation lasts more than 24 hours after instillation, and this effect depends on endogenous prostaglandins in rabbit eyes.

Because the effect of prostaglandins is markedly different among species, we performed a similar experiment in monkeys. Frequent ketamine anesthesia weakens cynomolgus monkeys, and therefore measurements were performed only once per day. Latanoprost significantly reduced IOP only in the treated eyes, consistent with previous results.[42] Furthermore, NB_{ONH} was not affected 4 hours after a single instillation, but gradually increased after once-daily administration for 5 and 7 days, only in the treated eyes, suggesting that the effects of latanoprost on ONH circulation accumulate with repeated instillations. Because latanoprost decreased IOP in monkeys, the observed NB_{ONH} may be attributable to the OPP increase. Therefore, the experiment was repeated in five of the eight animals after a 4-week interval, measuring the NB_{ONH} just before the morning instillation of latanoprost. The IOP level at 8 AM before instillation of latanoprost on the seventh experimental day decreased as expected, but NB_{ONH} did not change significantly from baseline. Differences between rabbits and monkeys in the effect on NB_{ONH} 24 hours after the 7-day instillation may be attributable to anatomic differences in distance between the conjunctival cul-de-sac and retrobulbar space around the optic nerve insertion[34,43] as well as differences in pharmacologic reaction to latanoprost among animal species.

Furthermore, the increase in the NB_{ONH} in the first experiment 4 hours after instillation on the eighth experimental day was not observed when indomethacin was intravenously injected 30 minutes before the measurement. Taken together, 7-day once-daily latanoprost instillation increased NB_{ONH} in the monkeys 4 hours, but not 24 hours, after the instillation, and this effect was probably not dependent on the IOP reduction and was significantly suppressed by intravenous indomethacin.

The above findings were consisted with those in rabbits, suggesting that the increase in NB_{ONH} observed in monkeys may also be attributable to local effects of latanoprost mediated by endogenous prostaglandin. Although it is not known whether a low concentration of latanoprost affects the endogenous prostaglandins system, a low concentration of $PGF_{2\alpha}$, a natural FP agonist, (2×10^{-8} to 1×10^{-7} M) reportedly exerts vasodilatory effects through PGI_2 in canine uterine arteries.[15]

In the human experiment, the result was essentially the same as in the monkey experiment, although general anesthesia may have partly modified the result in monkeys. NB_{ONH} just before the morning instillation of latanoprost on the eighth experimental day did not change significantly from that on the first experimental day (baseline) in both eyes, whereas the IOP at that time point was significantly reduced only in the treated eye. After the instillation of latanoprost, NB_{ONH} increased only in the treated eye. Seong et al.[25] reported that a single instillation of latanoprost did not significantly change the ONH and peripapillary retinal blood flow measured with HRF in normal humans. There are several differences between the present study and that of Seong et al.[25] In the present study, effects of 7-day once-daily instillation were examined, whereas in their study effects of a single instillation were studied. By the confocal principle of the scanning laser system, circulation in the superficial layer of retina or ONH is mainly measured with HRF,[44] whereas circulation in the deeper tissues significantly contributes to results with the laser speckle method.[26,32,33,45] Although this difference exists, the current result in monkeys, in which a single instillation did not significantly increase NB_{ONH}, is consistent with the results of Seong et al.[25]

The present findings in rabbit, monkey, and human eyes suggest that topical latanoprost increases blood velocity in the ONH or dilates the vessels supplying the ONH circulation, not because of a secondary effect of IOP reduction, but because of its pharmacologic effect on the vessels. In the normal eye, it should be very difficult for the instilled latanoprost to reach the ONH through the choroid or vitreous. It is not known whether latanoprost at a pharmacologically active concentration can penetrate to the retrobulbar space around the ONH where short posterior ciliary arteries exist, or whether a low concentration of latanoprost can stimulate endogenous prostaglandins. There is some evidence, however, suggesting that a small amount of the topically instilled drug can reach the retrobulbar parts of the eye and influence the ONH circulation, at least in rabbits,[46] and that a low concentration of $PGF_{2\alpha}$, a natural FP agonist, can stimulate endogenous PGI_2.[15] Further elucidation of the underlying mechanism is necessary.

In the present study, we did not examine how long the NB_{ONH}-increasing effect continued in normal humans, and no experiments were performed in aged subjects or patients with glaucoma. The potential of latanoprost to influence the ONH circulation independent of an IOP reduction deserves further investigation in healthy individuals as well as in aged subjects and patients with glaucoma.

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