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 administered latanoprost on optic nerve head (ONH) circulation in Dutch rabbits, cynomolagus monkeys, and normal humans.

METHODS. The ONH tissue blood velocity (NBONH) 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, NBONH was measured for 90 minutes after a single instillation and before and after a 7-day once-daily instillation regimen. In monkeys, NBONH 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 NBONH change was also studied in rabbits and monkeys. In humans, the time-course changes in NBONH 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 NBONH 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 NBONH 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.

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 (Imean) and the speckle intensity for successive scans of the image speckles at the pixels on the sensor plane was calculated, and the ratio of Imin to this difference was defined as normalized blur (NB). NB is essentially equivalent to the reciprocal of speckle contrast described by Ficher and Brier28,29 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 NBav. 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, NBav 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 NBav. In human experiments, NBav was measured for 5 seconds, during which no eye apparent movements were observed, and the average over three heartbeats was adopted as NBav.

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 sphygmonanometer (BP-9000; Softron, Tokyo, Japan) and in monkeys in the forearm with an infant sphygmonanometer (SP-8800; Nihonkoden, Tokyo, Japan). The mean blood pressure (BPav) was calculated according to the formula: BPav = BPd + 1/3(BPs − BPm), where BPd and BPm are diastolic and systolic brachial blood pressure, respectively. In monkeys, arterial O2 saturation (SaO2), and body temperature were monitored using a pulse oxygen meter (OLV-1200; Nihonkoden) and thermometer (Thermopit IT-500M; Nipro, Osaka, Japan).

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

**Drugs**

Latanoprost (13,14-dihydro-17-phenyl-18,19,20-trinor-PGF2α, isopropylster) 0.005% ophthalmic solution (Xalatan; Pharamcia, Uppsala, Sweden) was used and the vehicle solution was made in the laboratory according to the published data 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, NBav 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 NBav measurement. Latanoprost (30 μl) was instilled into one randomly chosen eye and vehicle into the other eye. NBav 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.

**Seven-Day Instillation**

On the first experimental day, after general anesthesia and pupil dilation, the NBav 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 NBav, 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 administered 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.

On the first experimental day, after general anesthesia was induced by ketamine hydrochloride (Ketal; Sankyo, Tokyo, Japan) at a dose of 8 to 10 mg/kg intramuscularly, and pupil dilation was induced with one drop of tropicamide in both eyes. The NBav, IOP, blood pressure, pulse rate, SaO2, 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 NBav 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 NBav is not suited for direct interindivid...
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**RESULTS**

**Rabbit Experiments**

A preliminary experiment revealed that coefficients of reproducibility of NBONH measurements at 30-, 60-, and 90-minute 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 NBONH 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, NBONH significantly increased by 13% to 16% from baseline at 30, 60, and 90 minutes after a single instillation (P = 0.055). In the vehicle-treated eyes, the NBONH did not significantly change. Bilateral differences in the ratio were also significant at 30, 60, and 90 minutes (P = 0.055).

In the indomethacin group (n = 8), no significant changes in the NBONH 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 NBONH increased significantly by approximately 15% at 30, 60, and 90 minutes (P = 0.055), whereas the NBONH in the vehicle-treated eyes remained almost unchanged.

In the fluorescein group (n = 8), fluorescein concentration in the anterior chamber was 1.78 ± 0.37 × 10⁻⁷ g/ml in the latanoprost-treated eyes and 1.70 ± 0.34 × 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 NBONH 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 1.** Time course of changes in NBONH 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 NBONH to baseline, with a bar denoting SE. *P < 0.05 by Wilcoxon signed rank test with Bonferroni correction for the bilateral difference.

**FIGURE 2.** Time course of changes in NBONH 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 NBONH 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.
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\textsubscript{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 $= \frac{2}{3}BP_m - IOP$.\textsuperscript{35} In the latanoprost-treated eyes on the fifth and eighth experimental days, IOP was significantly lower (paired \textit{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\textsubscript{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\textsubscript{ONH} significantly increased from baseline on the eighth day by 19.0% ($P = 0.035$), compared with 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).

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\textsubscript{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 2960 Ishii et al.

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

<table>
<thead>
<tr>
<th>Experimental Day</th>
<th>Baseline</th>
<th>2</th>
<th>5</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP\textsubscript{m} (mm Hg)</td>
<td>70.6 ± 7.4</td>
<td>65.4 ± 7.1</td>
<td>67.9 ± 7.9</td>
<td>71.5 ± 7.5</td>
</tr>
<tr>
<td>(beats/min)</td>
<td>159.9 ± 12.1</td>
<td>164.5 ± 9.9</td>
<td>171.8 ± 8.3</td>
<td>153.8 ± 7.4</td>
</tr>
<tr>
<td>BT(°C)</td>
<td>36.6 ± 0.4</td>
<td>36.4 ± 0.5</td>
<td>36.5 ± 0.4</td>
<td>36.5 ± 0.5</td>
</tr>
<tr>
<td>SaO\textsubscript{2}</td>
<td>99.1 ± 0.4</td>
<td>98.7 ± 0.7</td>
<td>98.4 ± 0.8</td>
<td>98.9 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM ($n = 8$). BP\textsubscript{m}, mean arterial blood pressure; BT, body temperature; SaO\textsubscript{2}, saturation of arterial O\textsubscript{2}.

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

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

**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 \textit{t}-test with Bonferroni correction for the bilateral difference.
measurement 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 NBONH between the latanoprost- and vehicle-treated eyes, averaging 18.2 ± 2.58 and 20.12 ± 2.11 (n = 11) at 9 AM before instillation. NBONH did not change significantly during the first day of the experiment in either eye. Before instillation of latanoprost on the eighth experimental day, the NBONH did not change significantly from that obtained before instillation on the first experimental day, but 180 minutes after instillation, NBONH significantly increased by 26% (P = 0.031) and tended to increase at 90 and 270 minutes (P = 0.051, 0.011), 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 = 0.003, 0.015 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, NBONH and blood flow rate determined using the hydrogen gas clearance method (in which a hydrogen electrode is inserted into the ONH tissue to assess the effective depth of sampling in the ONH tissue) was also significant at 90, 180, and 270 minutes (P = 0.013, 0.011, Fig. 6, right).

NBONH and blood flow rate, determined using the hydrogen gas clearance method,32,33 These results suggest that NBONH, 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

\[ F = \frac{2}{3} \pi \times D^4 \times \frac{\Delta P}{h} \]

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

\[ V_{\text{mean}} = \frac{1}{32} \times \frac{\Delta P}{h} \]  

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 NBONH. Therefore, the increase in the NBONH 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<sub>ONH</sub> increased not only after instillation, but also before instillation (24 hours after the last instillation). Moreover, an increase in NB<sub>ONH</sub> 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<sub>ONH</sub> 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<sub>ONH</sub> 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<sub>ONH</sub> 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<sub>ONH</sub> did not change significantly from baseline. Differences between rabbits and monkeys in the effect on NB<sub>ONH</sub> 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 as well as differences in pharmacologic reaction to latanoprost among animal species.

Furthermore, the increase in the NB<sub>ONH</sub> 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<sub>ONH</sub> 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 consistent with those in rabbits, suggesting that the increase in NB<sub>ONH</sub> 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 prostaglandin system, a low concentration of PGF<sub>2α</sub>, a natural FP agonist, can stimulate endogenous PGI<sub>2</sub>.15 Further elucidation of the underlying mechanism is necessary.

In the present study, we did not examine how long the NB<sub>ONH</sub>-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.

References

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