Topical Nipradilol: Effects on Optic Nerve Head Circulation in Humans and Periocular Distribution in Monkeys

Ken Mizuno, Takashi Koide, Naobiro Saito, Mikio Fujii, Miyuki Nagabara, Atsuo Tomidokoro, Yasubiro Tamaki, and Makoto Araie

PURPOSE. To investigate the effects of topical nipradilol on blood velocity in the optic nerve head (ONH) in normal humans and the ocular and periocular distribution of topically instilled nipradilol in monkeys.

METHODS. In normal humans, 0.25% nipradilol was instilled in one eye and vehicle in the other twice daily for 7 days, and blood velocity in the ONH was measured by the laser speckle method. In monkeys, after a single instillation of 1% [14C]nipradilol in one eye, distribution of radioactivity was evaluated by whole-head autoradiography.

RESULTS. Twice-daily 7-day instillation of nipradilol temporarily but significantly increased human ONH blood velocity, in the ipsilateral eye compared with the contralateral eye (P = 0.005), independent of a reduction in the intraocular pressure. In monkeys, equivalent nipradilol concentration in the periocular tissue around the optic nerve insertion was higher on the ipsilateral side than on the contralateral side (140 ± 25 ng/g and 42 ± 10 ng/g, P = 0.022, n = 5). Radioactivity was higher in the periocular tissue behind the equator than around the optic nerve insertion on the ipsilateral side (P = 0.004), but not on the contralateral side. The equivalent nipradilol concentration in the ipsilateral posterior retina-choroid was 636 ± 92 ng/g, which was significantly higher than that on the contralateral control side (521 ± 92 ng/g, P < 0.001).

CONCLUSIONS. The ipsilateral increase in ONH blood velocity induced by topical nipradilol in humans was attributed to drug that penetrated locally. Whole-head autoradiographic study suggests that topically instilled nipradilol can rapidly reach the posterior periocular tissue at pharmacologic concentrations.


Glaucoma is a disease that primarily damages the optic nerve head (ONH). One of the major risk factors in glaucoma is chronically elevated intraocular pressure (IOP). However, reports of the relation between a subtype of glaucoma and low blood pressure, migraine, and optic disc hemorrhage or findings on the favorable effect of a Ca2+ channel blocker on the visual field suggest that not only an increase in IOP but disorders of the systemic and/or local circulation are involved in the development of glaucomatous ONH damage.

There are two ways for antiglaucoma agents to modify circulation in the ONH. One is to increase ocular perfusion pressure (OPP) while decreasing IOP. It is not known, however, whether a chronic increase in OPP in fact causes a chronic change in blood flow in the ONH. The other way is to effect direct, drug-mediated vascular dilatation. Several in vitro studies are available on the vasodilatory effects of antiglaucoma agents. However, it is not known whether a topically instilled drug reaches the ONH or the vessels supplying the ONH at effective concentrations.

The ophthalmic artery branches into the central retinal artery, posterior ciliary arteries, and anterior ciliary arteries. Short posterior ciliary arteries, separate from the posterior ciliary artery, penetrate the eye wall around the insertion of the optic nerve and are essential in supplying blood to the ONH. A topically instilled drug may be unlikely to reach the ONH at pharmacologically effective concentrations: however, it could reach the posterior extraocular tissues surrounding the optic nerve at pharmacologic levels, the drug could modify the blood flow to the ONH.

Nipradilol (3,4-dihydro-8-(2-hydroxy-3-isopropylamino)pro-poxy-3-nitroxy-2H-1-benzopyran, molecular weight: 326.35) is a newly developed antiglaucoma ophthalmic agent that has nonselective β-receptor and selective α1-receptor blocking properties and a nitric oxide (NO) donor action. Topical instillation of 0.25% nipradilol lowers IOP as effectively as 0.5% timolol with a less systemic β-blocking effect. It has been found to increase ONH blood velocity in experimental animals, which may be attributed mainly to drug that penetrates locally.

In this study, we examined whether topically instilled nipradilol also influences blood velocity in the ONH in eight normal humans as a pilot study. Because the result suggested that the effect was attributable to local penetration of the drug, we examined the ocular and periocular distribution pattern of nipradilol after topical instillation, by analysis of whole-head autoradiographs in monkeys.

METHODS

The study of human ONH blood velocity was approved by the Ethics Review Committee of the University of Tokyo School of Medicine and the tenets of the Declaration of Helsinki were observed. Before admission into the study, each subject signed a written consent form after the nature of the study had been fully explained and routine eye examinations had been performed. Animal experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic Vision Research.

Effects on ONH Circulation in Humans

Eight young volunteers (20–24 years of age) with no history of smoking, who had neither systemic nor ocular disease and only mild refractive errors, participated in the study. On the day on which measure-
ments were performed, the participants did not drink coffee, tea, or alcohol and engaged in no strenuous exercise. The ONH blood velocity was measured by the laser speckle method and evaluated as a normalized blur (NB\text{corr}) value, details of which have been reported elsewhere.\textsuperscript{27–30}

In this system, a fundus camera equipped with a diode laser (wavelength, 808 nm) is used. The target area of the fundus on which the laser beam is focused is visualized by means of an infrared charge-coupled device (CCD) camera. The scattered laser light is recorded on a sensor (100 × 100 pixels, BASIS type; Canon, Tokyo, Japan) corresponding to the 1.06 × 1.06-mm field (45° visual angle) or 0.72 × 0.72-mm field (30° visual angle) of the human fundus on which the speckle pattern appears. The difference between the average speckle intensity (I\text{mean}) and the intensity of successive scans of speckles is calculated. The ratio of I\text{mean} to this difference is defined as NB, an approximate equivalent of the reciprocal of the speckle contrast\textsuperscript{31–32} indicating blood velocity in the tissue. The NB is calculated by the logic board every 0.125 seconds for seven successive seconds, divided into 50 color-coded levels, and displayed on a color monitor, showing a two-dimensional variation of NB over the field of interest.\textsuperscript{53} The average NB (NB\text{avg}) in any rectangular field of interest displayed on a color map can be determined, as well as the change in NB\text{avg} during 7 seconds.

Subjects were asked to focus on a target light in a dimmed measurement room 90 minutes after topical instillation of 0.4% tropicamide for mydriasis. The image speckles of the measurement field in the temporal ONH (0.72 × 0.72 mm; 30° visual angle), were recorded, and the NB of the largest rectangular field free of visible surface vessels was calculated over three cardiac cycles in which fixation was satisfactory to obtain the mean NB\text{ONH} of the ONH (NB\text{corr}). The size of the measurement field varied among individual subjects because of the necessity to avoid surface vessels and ranged from 0.14 × 0.22 mm (20 × 30 pixels) to 0.29 × 0.43 mm (40 × 60 pixels). Movement of the subject’s eye during measurement was observed as previously described.\textsuperscript{53}

On the first experimental day (day 0), bilateral NB\text{ONH} and IOP, brachial arterial blood pressure (BP), and pulse rate (PR) were measured at 11:00 AM, after bilateral mydriasis with 0.4% tropicamide at 9:30 AM. IOP was measured with a Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). BP and PR were measured with an automatic sphygmomanometer (BP-203RV II; Colin, Tokyo, Japan). All measurements were completed in approximately 5 minutes. Immediately after the data were obtained, 1 drop (50 \muL) of vehicle was instilled in both eyes. Measurements were repeated 45, 90, and 180 minutes after this instillation (at 11:45 AM, 12:30 PM, and 2:00 PM). Five minutes after each measurement, 0.4% tropicamide was instilled bilaterally. On the following day (day 1), 1 drop (50 \muL) of 0.25% nipradilol was instilled in one randomly chosen eye of each subject, and vehicle was instilled in the contralateral eye. Subjects were masked to the laterality of the nipradilol treatment. From this day on, twice-daily instillations (11 AM and 11 PM) of 0.25% nipradilol and vehicle into the chosen eyes were continued for 7 days. On days 1 and 7, NB\text{ONH}, IOP, BP, and PR were recorded at 11:00 AM just before the morning instillation, as just described. Immediately thereafter, the morning instillation was performed, and measurements were repeated at 11:45 AM, 12:30 PM, and 2:00 PM as on day 0.

The NB\text{ONH}, and IOP, BP, and PR were recorded by separate investigators (AT, YT) blinded to the treatment of each eye. The results of the laser speckle measurement were digitally stored on magneto-optical disks as color maps from which NB\text{ONH} was determined by a blinded investigator (MN).

Mean brachial arterial blood pressure (BP\text{m}) was determined by the following formula

$$BP_m = BP_s + \frac{1}{3}(BP_d - BP_s) \quad (1)$$

where BP\text{s} and BP\text{d} are systolic and diastolic pressures. OPP was also calculated

\text{Distribution of Topically Instilled Nipradilol}

\textsuperscript{[\textsuperscript{14}\textsuperscript{C}] nipradilol (Code CFQ11032, radiochemical purity 98%, specific radioactivity 1.55 MBq (43 \muCi/mg) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, UK).

Seven male cynomolgus monkeys (Macaca fascicularis, 5 to 8 years old; Japan SLC, Shizuoka, Japan) were used. One monkey was used to confirm that there was no unspecific reaction not attributable to radioactivity in the present method of autoradiography, one was used for autoradiography at 15 minutes, and five were used for autoradiography at 60 minutes after the instillation.Autoradiographic quantitative analysis was performed at 60 minutes after the instillation. Each monkey was placed supine under ketamine (ketamine hydrochloride; Sankyo, Tokyo, Japan) anesthesia.\textsuperscript{[\textsuperscript{3}\textsuperscript{C}] nipradilol (1%, 100 \muL, 1.5 MBq [41 \muCi] per dose) was instilled into the right eye, and the left eye was left untouched. Fifteen or 60 minutes after the instillation, monkeys were killed by the intravenous injection of pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL). Blood was obtained from the right femoral vein just before the pentobarbital injection. The method for autoradiography was basically the one described by Ullberg.\textsuperscript{34} Briefly, the head was immersed in the mixture of hexane and solid carbon dioxide for 20 minutes. Immediately thereafter, the monkey was decapitated, and the head was stored overnight at −15°C in a container of cryomicrotome (Cryomicrocut; Leika Microsystems GmbH, Nussloch, Germany) for the evaporation of hexane. After the removal of fur, the head was mounted in a 3% carboxymethyl cellulose (CMC) gel and cut in 30-\muM sections with a cryomicrotome (Leika Microsystems). The following two sections were used for autoradiography: one cut just above the temporal side of the lens and the other cut through the ONHs and midlines. Each section was covered with thin film (4 \muM Diafoil; Hoechst, Tokyo, Japan) and exposed to an imaging plate (BAS-SR; Fuji Photograph Film, Tokyo, Japan) for 72 hours to develop an autoradiograph that was visualized with image analyzer (Fujix BAS2500 Bioimaging Analyzer, Fuji Photograph Film). This device can detect the radioactivity concentration in each section as the intensity of the photo-stimulated luminescence and can visualize the gradation of radioactivity concentration as a color image. The scanning conditions were as follows: gradation 65,536, resolution 50, latitude 5, and sensitivity 4,000.

In the anterior chamber, lens, vitreous, equatorial and posterior retina-choroid, and peripercular tissue behind the equator and around the optic nerve insertion, quantitative analysis of the \textsuperscript{[\textsuperscript{14}\textsuperscript{C}]} nipradilol concentration on treated and contralateral control sides at 60 minutes after instillation was performed on the sections cut through the ONH and midline. The positions of the equatorial and posterior retina-choroid were defined as the 2-mm length of the equator and just beside the ONH of the retina-choroid, respectively. Peripercular tissue behind the equator was defined as an approximately 1-mm\textsuperscript{2} equatorial triangular area between the lateral extracocular muscle and sclera. Peripercular tissue around the optic nerve insertion was defined as an approximately 2-mm\textsuperscript{2} area just beside the optic nerve insertion, avoiding an area with apparent higher radioactivity that is probably attributable to the extracocular muscle. Each position on the side of the treated eye is included in Figure 2G. The densitometric readings obtained from the autoradiographs by a masked investigator were converted into radioactivity by using standard curves developed on the imaging plate from the rat liver homogenate, as described later, and quantified by the following equation

$$\text{Concentration} = \frac{(PSL - BG)}{A} \quad (3)$$

where PSL is photo-stimulated luminescence, BG is background PSL, and A is the area of each tissue on the image analyzer.

Three Sprague-Dawley rats (7 weeks old, Japan Laboratory Animals, Tokyo, Japan) were used to make standard curves for quantitative determinations. Each rat was killed and the liver was isolated. The liver
between the two eyes in IOP was significantly lower than at the same time on day 0 (NBONH ratio) were calculated and compared between the two eyes. The logarithmic standard curve for the [14C]nipradilol concentration showed a very good correlation between the data obtained with the scintillation counter and the PSL data in the autoradiograph (Fig. 3). The [14C]nipradilol concentration in each tissue at 60 minutes after the instillation calculated by this correlation was summarized in Table 2. In the periocular tissues, the equivalent concentration of nipradilol was significantly greater in the n企radilol-treated than in the vehicle-treated side (Fig. 1I, P = 0.005, Wilcoxon signed rank test).

**Table 1.** Systemic and Ocular Parameters before and after Topical Application of Nipradilol or Vehicle

<table>
<thead>
<tr>
<th></th>
<th>0 minutes</th>
<th>45 minutes</th>
<th>90 minutes</th>
<th>180 minutes</th>
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<tbody>
<tr>
<td>BPm (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>82.3 ± 1.3</td>
<td>82.3 ± 1.6</td>
<td>80.0 ± 1.6</td>
<td>80.1 ± 1.5</td>
</tr>
<tr>
<td>Day 1</td>
<td>79.2 ± 1.9</td>
<td>79.8 ± 1.6</td>
<td>75.5 ± 2.7</td>
<td>77.8 ± 2.1</td>
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<tr>
<td>Day 7</td>
<td>75.3 ± 1.4</td>
<td>75.6 ± 2.0†</td>
<td>75.5 ± 1.7</td>
<td>78.1 ± 2.6</td>
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<tr>
<td>PR (beats/minute)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>70.5 ± 3.5</td>
<td>69.9 ± 3.3</td>
<td>68.1 ± 3.2</td>
<td>70.9 ± 2.6</td>
</tr>
<tr>
<td>Day 1</td>
<td>74.8 ± 5.0</td>
<td>74.1 ± 5.4</td>
<td>72.1 ± 5.0</td>
<td>74.0 ± 4.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>72.0 ± 3.6</td>
<td>71.5 ± 3.5</td>
<td>71.5 ± 4.0</td>
<td>72.5 ± 4.3</td>
</tr>
<tr>
<td>OPP (mmHg)</td>
<td></td>
<td></td>
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<tr>
<td>Day 0</td>
<td>43.5 ± 0.8</td>
<td>44.8 ± 1.3</td>
<td>43.5 ± 1.4</td>
<td>43.8 ± 1.1</td>
</tr>
<tr>
<td>Day 1</td>
<td>42.7 ± 1.2</td>
<td>44.6 ± 1.1</td>
<td>44.0 ± 1.9</td>
<td>43.2 ± 1.2</td>
</tr>
<tr>
<td>Day 7</td>
<td>41.3 ± 1.3</td>
<td>42.0 ± 1.7</td>
<td>41.7 ± 1.4</td>
<td>43.4 ± 1.8</td>
</tr>
<tr>
<td>Day 0 (vehicle)</td>
<td>43.9 ± 0.7</td>
<td>44.6 ± 1.3</td>
<td>43.0 ± 1.4</td>
<td>43.7 ± 1.2</td>
</tr>
<tr>
<td>Day 1 (vehicle)</td>
<td>42.9 ± 1.1</td>
<td>43.6 ± 1.2</td>
<td>42.5 ± 1.7</td>
<td>42.7 ± 1.4</td>
</tr>
<tr>
<td>Day 7 (vehicle)</td>
<td>40.6 ± 1.1</td>
<td>40.8 ± 1.6</td>
<td>39.5 ± 1.5</td>
<td>41.6 ± 1.8</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM.

*P < 0.05, †P < 0.01 versus same time on day 0 (paired t-test with Bonferroni correction). n = 8.

was homogenized and mixed with four concentrations (100, 1000, 10,000, and 100,000 Bq/g) of [14C]nipradilol. These standard samples were dissolved in 2 mL of a tissue solubilizer (Soluene-350; Perkin Elmer [formerly Packard Instruments], Wellesley, MA). Concentration of [14C]nipradilol in each standard sample was measured by liquid scintillation counter and mounted in blocks of CMC to make standard curves.

**Statistical Analysis**

All data are expressed as the mean ± SEM. In the human experiment, BPm, PR, OPP, and IOP were compared with data of the same time point on day 0 by paired t-test with Bonferroni correction. NB, a quantitative index of blood flow, is not an absolute value, and therefore it is not advisable to compare directly the NB values obtained from both eyes. Thus, the data obtained in each eye on days 1 and 7 were compared with data at the same time on day 0 by paired t-test with Bonferroni correction, and the ratio of the data obtained on days 1 and 7 to those obtained at the same time on the same eye on day 0 (NBONH ratio) were calculated and compared between the two eyes by the Wilcoxon signed rank test. In the monkey study, a paired t-test was used.

**RESULTS**

**Effects on the Human ONH Blood Velocity**

On day 0, there was no significant difference in the systemic parameters, IOP and NBONH throughout the measurement period (Table 1, Figs. 1A, 1B, 1C, 1F, 1G). On day 1, systemic parameters remained unchanged (Table 1), and the difference between the two eyes in IOP was significant at 90 minutes (Fig. 1D, P = 0.040, paired t-test). NBONH on the n企radilol-treated side tended to be increased at 90 minutes (Fig. 1H, P = 0.010, Wilcoxon signed rank test). On day 7, BPm at 0 and 45 minutes was significantly lower than at the same time on day 0 (Table 1, P = 0.012 and 0.008 for 0 and 45 minutes, respectively, paired t-test with Bonferroni correction). Other systemic parameters were unchanged. On day 7, IOP at 0 minutes in the n企radilol-treated side was significantly lower than at the same time on day 0 (Fig. 1B, P = 0.015; paired t-test with Bonferroni correction), and the difference between the two eyes was also significant at 90 and 180 minutes (Fig. 1E, P = 0.001 and P = 0.006, respectively; paired t-test). NBONH at 90 minutes on the n企radilol-treated side was significantly higher than at the same time on day 0 (Fig. 1G, P = 0.027, paired t-test with Bonferroni correction), and the NBONH ratio at 90 minutes was significantly greater in the n企radilol-treated than in the vehicle-treated side (Fig. 1I, P = 0.005, Wilcoxon signed rank test).

**Distribution of Topically Instilled Nipradilol**

Figure 2A shows the frozen sections of a monkey’s head obtained through the temporal side of the lens. Both lenses could be seen just below the surface of the section. The section through the ONH and the midline of both eyes is shown in Figure 2B.

There were no nonspecific reactions that were not attributable to radioactivity in the present method. Autoradiographs corresponding to Figures 2A and 2B, 15 and 60 minutes after instillation are shown in Figures 2C and 2E, and 2D and 2F, respectively. Fifteen minutes after instillation, the level of radioactivity was highest in the cornea and conjunctiva, followed by the anterior chamber, iris, ciliary body, lens, and retinal-choroid in the instilled eye. Radioactivity was also observed in the iris, ciliary body, and retina-choroid of the contralateral eye. Outside the eyeball of the instilled side, radioactivity was detected in the extraocular muscles, periocular tissues around the equator, behind the equator, and around the optic nerve insertion within the orbit. A similar distribution pattern was observed at 60 minutes after the instillation, whereas the radioactivity in the retina-choroid or periocular tissues was apparently higher than that at 15 minutes.
FIGURE 1. Effects of nipradilol and vehicle on IOP and NBONH in normal human eyes. Symbols and vertical bars are the mean ± SEM. (A) IOP of vehicle-treated eye on day 0 (○), day 1 (∆), and day 7 (◇). (B) IOP of nipradilol-treated eye on day 0 (●), day 1 (▲), and day 7 (◆). (C) IOP of vehicle- (○) and nipradilol (●)-treated eye on day 0. (D) IOP of vehicle- (∆) and nipradilol (▲)-treated eye on day 1. (E) IOP of vehicle- (○) and nipradilol (●)-treated eye on day 7. (F) NBONH of vehicle-treated eye on day 0 (○), day 1 (∆), and day 7 (◇). (G) NBONH of nipradilol-treated eye on day 0 (●), day 1 (▲), and day 7 (◆). (H) NBONH ratio of vehicle- (○) and nipradilol (●)-treated eye on day 7. *P < 0.05 vs. 0 minutes on day 0 (paired t-test with Bonferroni correction). †P < 0.05, ‡P < 0.01, and §P < 0.01 versus same time of vehicle-treated eye (paired t-test). ¶P < 0.05 versus same time of day 0 (paired t-test with Wilcoxon signed-rank test). #P < 0.01 versus same time of vehicle-treated eye (Wilcoxon signed-rank test). n = 8.
contralateral control side (212 ± 21 ng/g, \( P = 0.03 \), and 521 ± 92 ng/g, \( P < 0.001 \), paired \( t \)-test). On the instilled side, the equivalent concentration of nipradilol tended to be higher in the equatorial retina-choroid than in the posterior retina-choroid (\( P = 0.094 \)), whereas that in the former was significantly lower than that in the latter on the contralateral control side (\( P = 0.026 \)).

**DISCUSSION**

The penetration depth of a near-infrared laser (wavelength, 811 nm) in the cat optic nerve\(^{35} \) and the results of previous studies in which the relative change in NB\(_{ONH} \) in rabbits was compared with that in blood flow, determined using the hydrogen gas clearance method under various conditions\(^{20,36} \).
suggests that the NB correlates well with the blood flow rate in the ONH, at least in the rabbit eye.

In the human study, topical instillation of nipradilol caused a transient, but significant increase in the ipsilateral ONH blood velocity after 7 days of twice-daily instillation. Moreover, at 0 and 180 minutes on day 7, IOP significantly decreased, but NB_{ONH} showed no significant change (Figs. 1B, 1G, 1I). These findings indicate that the increase in ONH blood velocity induced by topical nipradilol in normal humans was not a secondary effect accompanied by a decrease in IOP in the ipsilateral eye. This effect is probably attributable to the vasodilative action of the nitradiol that penetrated locally. Kanno et al. reported that topical nitradiol increases the ONH blood velocity in normal humans by twice-daily instillation by whole-head autoradiography.

It may be interesting to estimate the minimum number of subjects needed to confirm the result of the present pilot study with a power of 0.8 (β = 0.2) and an α error of 0.05, in patients who normally use topical nitradiol in both eyes, and a randomized, double-masked, crossover study design. We previously evaluated intra-individual variation of NB_{ONH} taken from the same site of the ONH at intervals of 3 weeks in normal volunteers who received twice-daily instillation of vehicle eye drops. The result indicated that a 10% change in the NB_{ONH} would be detected with 20 subjects.

In the second part of the study, we examined the distribution of nitradiol after topical instillation by whole-head autoradiography in the monkey. In the anterior parts of the eye, radioactivity was high, as expected. Relatively high radioactivity was found in the posterior retina-choroid, not only in the instilled eye but also in the fellow control eye. The apparently higher radioactivity in the retina-choroid in the control eye than in the subdermal tissues, extraocular muscles of the untreated side, or blood suggests that nitradiol or its metabolites from the general circulation were bound to and accumulated in the uveal pigment.

Radioactivity in the posterior pericircular tissue was found to be unexpectedly high, even 15 minutes after the instillation. Because radioactivity in the pericircular tissue around the optic nerve insertion appeared to be lower than that in the pericircular tissue behind the equator on the untreated side but higher than that on the untreated side, local penetration of nitradiol from the conjunctival cul-de-sac through the pericircular tissues seemed plausible. To confirm these rather qualitative findings, an autoradiographic quantitative analysis 60 minutes after the instillation was performed. Results of the quantitative analysis and the equivalent nitradiol concentration directly calculated from radioactivity are shown in Table 2. The concentration of nitradiol thus estimated in the anterior chamber in the treated eye was several times higher than, but that in the lens was comparable to, that obtained in our previous study, in which radioactivity was measured in rabbit eyes after instillation of nitradiol.

The apparently higher radioactivity in monkey eyes than in rabbit eyes may be explained by a difference in the corneal epithelial permeability between the animals used and/or a difference in the methodology. In the posterior retina-choroid, radioactivity was significantly higher on the treated side than in the fellow control eye, suggesting that part of the radioactivity in the posterior retina-choroid of the instilled eye is attributable to the local penetration of the drug. This finding is also compatible with our previous results in rabbits. The higher radioactivity in the equatorial retina-choroid and negligible radioactivity in the vitreous may not exclude diffusion of the drug from the anterior to posterior choroid.

Table 2. Calculated Nitradiol Concentration in Each Tissue of the Monkey

<table>
<thead>
<tr>
<th>Treated Side</th>
<th>Control Side</th>
</tr>
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<tbody>
<tr>
<td>Anterior chamber</td>
<td>20534 ± 4607*</td>
</tr>
<tr>
<td>(65 ± 14)</td>
<td>34 ± 12</td>
</tr>
<tr>
<td>Lens</td>
<td>710 ± 193†</td>
</tr>
<tr>
<td>(2.18 ± 0.59)</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>Vitreous</td>
<td>N.D.</td>
</tr>
<tr>
<td>(N.D.)</td>
<td>(0.07 ± 0.02)</td>
</tr>
<tr>
<td>Equatorial retina-choroid</td>
<td>1655 ± 434‡</td>
</tr>
<tr>
<td>(5.07 ± 1.33)</td>
<td>212 ± 21*</td>
</tr>
<tr>
<td>Posterior retina-choroid</td>
<td>636 ± 925*</td>
</tr>
<tr>
<td>(1.95 ± 0.28)</td>
<td>521 ± 92</td>
</tr>
<tr>
<td>Periocular tissues behind the equator</td>
<td>231 ± 30*</td>
</tr>
<tr>
<td>(0.71 ± 0.09)</td>
<td>41 ± 10</td>
</tr>
<tr>
<td>Periocular tissues around the optic nerve insertion</td>
<td>140 ± 25*</td>
</tr>
<tr>
<td>(0.43 ± 0.08)</td>
<td>42 ± 10</td>
</tr>
<tr>
<td>Blood</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>(0.05 ± 0.02)</td>
<td></td>
</tr>
<tr>
<td>Plasma</td>
<td>14 ± 4</td>
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<tr>
<td>(0.04 ± 0.01)</td>
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</tbody>
</table>

* P < 0.05, † P < 0.05, ‡ P < 0.05, § P < 0.01, ¶ P < 0.05 versus control side (paired t-test). * P < 0.05 vs. control side (paired t-test). ** P < 0.01 vs. treated side of posterior pericircular tissues. n = 5.
metabolism of instilled nipradilol is not largely different between cynomolgus monkeys and rabbits, 60 minutes after the instillation approximately 50% of the lateral difference in radioactivity is attributable to unchanged nipradilol. That is, approximately 0.175 μM (0.5 × (1.95–1.60) μM) nipradilol is thought to have reached the posterior retina-choroid by local, but yet undefined routes. As inferred from the much higher radioactivity in the posterior retina-choroid of the fellow control eye than in the plasma, however, most of the nipradilol is probably bound to uveal pigment and not pharmacologically active and the free nipradilol concentration in the posterior retina-choroid is unknown in the present experimental design. Some of the previous reports refer to penetration of topically instilled antiglaucoma agents to posterior parts of the eye. After a single instillation in pigmented rabbits, averaged concentrations of timolol,47 befunolol,59 carteolol,48 or bunolol60 in the ipsilateral equatorial and posterior retina-choroid were reportedly on the order of 1/1000 of those instilled, which is compatible with the present result obtained with nipradilol in monkeys. As shown in the present result (Table 2), most of the radioactivity may be attributable to the drug from systemic circulation and bound to uveal pigment. On the contrary, topically applied sultamidines65 or latanoprost60 reportedly show no direct access to the ipsilateral retina. This discrepancy may be at least partly attributable to chemical difference in the compounds.

In the pericircular tissues around the optic nerve insertion, radioactivity was also significantly higher on the treated side than the untreated side, and the higher radioactivity in the pericircular tissues behind the equator than around the optic nerve insertion (Table 2) suggests that this lateral difference is attributable to nipradilol which penetrated through the pericircular route from the conjunctival cul-de-sac. Approximately 0.15 μM (0.5 × (0.43–0.13) μM) nipradilol was thought to have reached the pericircular tissues around the optic nerve insertion by local penetration. This interpretability is compatible with the observation by Sposnol et al.51 that Tenon’s capsule accumulates betaxolol or timolol at much higher concentrations than intraocularly after long-term topical therapy. Although there have been no previous reports measuring the drug concentration in the posterior pericircular tissues after topical instillation, the drug concentration in the ipsilateral optic nerve may not be far from that in the ipsilateral pericircular tissue. It was reportedly on the order of 1/100 of that instilled within 1 hour after a single instillation for timolol,47 befunolol,47 carteolol,48 or levobunolol,52 which does not conflict with the present finding. The clinical dose and volume of nipradilol were 0.25% and 50 μL, respectively, whereas in this study we used 1% nipradilol and 100 μL to magnify the sensitivity. Thus, at least 0.02 μM (0.15 μM × 0.25%/1% × 50 μL/100 μL) of unchanged nipradilol is thought to reach the pericircular tissues around the optic nerve insertion at the clinical dose in the monkey eyes. Okamura et al.24 examined the vasodilating effect of nipradilol on isolated canine retinal central arteries and reported a dose-dependent relaxation at 0.001 to 10 μM. Thus, the nipradilol concentration in the pericircular tissues around the optic nerve insertion in this study may be high enough to dilate short posterior ciliary arteries.24

If we assume that the penetration pattern of nipradilol is similar between humans and monkeys, the concentration of nipradilol in the human ipsilateral pericircular tissues around the optic nerve insertion may be higher than 10⁻⁸ M. The temporal effect of ONH blood velocity of topical nipradilol in the ipsilateral human eye found in the present pilot study may be at least partly attributable to drug that penetrated through the pericircular route to the pericircular tissue around the optic nerve insertion. The clinical implications of nipradilol’s effect of increasing blood velocity in humans may be minor, in that the effect was brief. However, the results of this study indicate that topical drugs have the potential to influence the ONH blood velocity and/or posterior choroidal blood flow by local penetration and by affecting short posterior ciliary arteries. Other topical drugs that are vasoactive may also influence the fundus circulation by the same mechanism.

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References


