Effects of Topical Nipradilol, a \( \beta \)-Blocking Agent with \( \alpha \)-Blocking and Nitroglycerin-Like Activities, on Aqueous Humor Dynamics and Fundus Circulation

Mikiko Kanno, Makoto Araie, Ken Tomita, and Kimio Sawanobori

**Purpose.** To study the effects of nipradilol, a nonselective \( \beta \)-blocker with \( \alpha \)-blocking activity and nitroglycerin-like activity, on aqueous humor dynamics and optic nerve head (ONH) circulation in albino rabbits.

**Methods.** Experiments were carried out during the dark phase, in conscious rabbits conditioned to a schedule of alternating 12-hour periods of light and dark. The blood-aqueous barrier permeability and the aqueous flow rate were determined fluorophotometrically. The effect on outflow to general blood circulation and uveoscleral outflow were determined by using the fluorophotometric Dia-mox technique, and the effect on the uveoscleral outflow was further assessed by using the anterior chamber perfusion method. The ONH circulation was estimated by using the laser speckle method.

**Results.** Unilateral topical administration of 0.25% nipradilol solution lowered intraocular pressure (IOP) with relatively weak contralateral effects in a dose-dependent manner with a maximum reduction of 6 mm Hg and an effect duration of 6 hours. Twice-daily instillation for 14 days showed no attenuation of the effects. Single instillation of 0.25% nipradilol showed no significant effect on blood-aqueous barrier permeability and decreased aqueous flow rate in the treated eye (17%; \( P < 0.01 \)) and in the contralateral eye (9%; \( P < 0.05 \)). Nipradilol produced no significant effect on outflow facility to general blood circulation, whereas it substantially increased uveoscleral outflow.

**Conclusions.** Because of its ability to lower IOP and to increase uveoscleral outflow and optic nerve head circulation in rabbits, further studies are warranted to determine whether nipradilol has potential as an antiglaucoma agent in humans. (Invest Ophthalmol Vis Sci. 1998;39:736–743)

Several lines of evidence suggest that not only increased intraocular pressure (IOP), but also compromise of circulation in the optic nerve head (ONH) play causal roles in the development of glaucoma.\(^1\)\(^2\) Thus, a drug with beneficial effects on ocular circulation and on the ocular hypertensive effect may be a more useful antiglaucoma agent. Nipradilol is a nonselective \( \beta \)-blocker with \( \alpha \)-blocking activity, and nitroglycerin-like vasodilating activity, which is attributed to its nitroxy moiety (Fig. 1).\(^3\)\(^–\)\(^5\) Its in vitro \( \beta \)-blocking activity is approximately twice that of propranolol,\(^6\) its \( \alpha \)-blocking activity approximately one fifth that of phentolamine,\(^7\) and its nitroglycerin-like vasodilating activity approximately one fifth that of nitroglycerin.\(^8\) Some \( \alpha \)-blockers, or nitroglycerin, have been reported to reduce IOP in experimental animals or normal humans by increasing uveoscleral outflow or tonographic outflow or by reducing aqueous flow, for at least a short period.\(^6\)–\(^14\) A highly selective \( \alpha \)-blocker, bunazosin, is known to reduce IOP in patients with glaucoma by approximately 4 mm Hg for 1 year without showing tachyphylaxis.\(^15\) Thus, the \( \alpha \)-blocking and nitroglycerin-like activities of nipradilol may increase the ocular hypotensive effect because of its \( \beta \)-blocking activity, which is expected to reduce aqueous production. Furthermore, the \( \alpha \)-blocking and nitroglycerin-like vasodilating activities of nipradilol may give it an advantage when compared with other \( \beta \)-blockers, without having such effects on ocular circulation. In the first attempt to assess the potential of nipradilol as a future antiglaucoma agent, the effect of topical nipradilol on IOP, aqueous humor dynamics, and ONH circulation was studied in albino rabbits.

**Materials and Methods**

**Animals**

New Zealand albino rabbits weighing 2 to 2.5 kg were used regardless of their sex. Animals were conditioned to a schedule of alternating 12-hour periods of light (8 AM–8 PM) and dark (8 PM–8 AM) for at least 3 weeks before use. If not otherwise indicated, rabbits were conscious and experiments were carried out during the dark phase.\(^16\) Procedures used conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Drugs**

Ophthalmic solutions of 0.06%, 0.125%, 0.25%, 0.5% nipradilol and the vehicle solution were supplied by Kowa (Tokyo, Ja-
pan). Timolol maleate 0.5% ophthalmic solution was purchased from Banyu Pharmaceutical (Tokyo, Japan).

**Instruments**

The IOP was measured with a calibrated applanation pneumotonomograph (Alcon, Fort Worth, TX) in animals under topical anesthesia. Fluorophotometric measurements were made with a slit lamp fluorophotometer (Topcon, Tokyo, Japan). The ONH circulation was evaluated by using the diode laser-based laser speckle method, of which details have been reported elsewhere. All measurements were carried out by investigators masked to the treatment.

**MATERIALS AND METHODS**

**Effect of Topical Nipradilol on Intraocular Pressure**

At 11 AM, 20 μl vehicle or a solution of 0.06%, 0.125%, 0.25%, or 0.5% nipradilol was instilled in the right eye. Intraocular pressure was measured in both eyes before and 1, 2, 3, 4, 5, 6, and 7 hours after instillation. Animals were administered each solution at intervals alternated with a 1-week washout period. The order of application of the five solutions was randomly assigned in each rabbit.

In another group of rabbits, 20 μl 0.25% nipradilol was instilled in one eye and same volume of vehicle solution in the other eye twice-daily at 11 AM and 11 PM hours for 14 days. Intraocular pressure was measured at 2 PM under dim light on the 5, 10, and 14 days.

**Comparison of Nipradilol and Timolol**

At 11 AM one of the following was administered in the subjects' right eyes: 20 μl vehicle solution for nipradilol; 0.25% nipradilol; 0.5% timolol; 20 μl 0.5% timolol, followed in 5 minutes by the same volume of 0.5% timolol; or 0.5% timolol, followed in 5 minutes by the same volume of 0.25% nipradilol. The IOP in the right eye was measured before and 1, 2, 3, 4, 5, 6, and 7 hours after the instillation. Animals received each set of solutions at intervals alternated with a 1-week washout period. The order of application of the five sets of solutions was randomly assigned in each rabbit.

**Effect on Blood–Aqueous Barrier Permeability**

At 11 AM, 20 μl vehicle was instilled in both eyes. At 12 noon, 0.05 mg/kg 10% fluorescein solution (Alcon) was injected intravenously, and at 1 PM, fluorescein concentration in the anterior chamber was measured in both eyes. One week later, the same experimental procedures were repeated, except that 20 μl 0.25% nipradilol was instilled in one randomly chosen eye and the vehicle in the fellow eye.

**Effect on Aqueous Flow Rate**

Three experiments were carried out at intervals alternated with a 1-week washout period. In the first experiment, 20 μl 0.25% nipradilol was instilled in one randomly chosen eye and the same volume of the vehicle in the fellow eye at 11 AM. The IOP in both eyes was measured 1 hour before and just before the instillation and every hour for 7 hours after the instillation. In the second experiment, 10% fluorescein was instilled in both eyes several times at 1-minute intervals at 11 PM, and the conjunctival cul-de-sac was rinsed well with physiological saline. At 11 AM on the next day, 20 μl vehicle was instilled in both eyes, and the fluorescein concentrations in the cornea (Cf), calculated from the fluorescence intensity in the cornea as previously reported, were determined in the equation and assuming Pcv of saline. At 11 AM the next day, 20 μl 0.25% nipradilol was instilled in the eye that received nipradilol in the first experiment, and the vehicle was instilled in the fellow eye. At the end of the third experiment, the entire aqueous humor was collected by paracentesis. From the total aqueous volume, the anterior chamber volume was calculated, assuming the anterior chamber volume accounts for 82% of the total volume of the aqueous in rabbits. The running average aqueous flow rate between 11 AM and 3 PM was calculated according to the Jones–Maurice method. That is

\[ F = 0.9(V_c g_{cv} A_c + V_v A_v) \]  \hspace{1cm} (1)

where \( F \) is the aqueous flow rate, \( A_c(A_v) \) the rate of decline of \( C_c(C_v) \); \( g_{cv} \) the mean of \( C_c/C_v \) during the experimental period used for calculation of \( F \); \( V_c \) the volume of the cornea, which was assumed to be 65 μl; and \( V_v \) the anterior chamber volume, which was determined as above.

**Effect on Outflow to General Blood Circulation and Uveoscleral Outflow**

The outflow facility to general blood circulation (Cgen) and uveoscleral outflow (F\(_\text{u}\)) were determined according to the method of Hayashi et al. If rabbits are treated with acetazolamide, which lowers the IOP by reducing \( F \), but without changing \( C_{gen} \), \( F_{u} \), or episcleral venous pressure (Pcv), \( C_{gen} \) is determined by

\[ C_{gen} = (F_{\text{before}} - F_{\text{after}})/(IOP_{\text{before}} - IOP_{\text{after}}) \]  \hspace{1cm} (2)

where \( F_{\text{before}} \) and \( F_{\text{after}} \) are \( F \) before and after acetazolamide administration, and IOP\(_{\text{before}} \) and IOP\(_{\text{after}} \) are IOP before and after acetazolamide administration, respectively. Using the value of \( C_{gen} \), determined in the equation and assuming \( F_{u} \) of
9 mm Hg\(^2\) and no significant effect of topical nipradilol on \(P_{ev}\). \(F_a\) is determined by

\[
F_a = F_{before} - C_{ev}(IOP_{before} - 9) \cdots \quad (3)
\]

or

\[
F_a = F_{after} - C_{ev}(IOP_{after} - 9) \cdots \quad (4)
\]

In the first experiment, fluorescein was instilled at 11 PM as described earlier and 20 \(\mu\)l vehicle was instilled in both eyes at 11 AM and 2 PM on the next day. Fluorophotometric measurements in the cornea and anterior chamber were carried out every hour from 11 AM to 6 PM, and the IOP was measured at 12 noon, 2 PM, 4 PM, and 6 PM in both eyes. Just after the 2 PM fluorescentophotometric and IOP measurements, 50 mg/kg acetazolamide was injected intravenously. \(F\) before acetazolamide administration was calculated from fluorophotometric measurements between 11 AM and 2 PM and \(F\) after acetazolamide administration from measurements between 3 PM and 6 PM. The IOP before acetazolamide administration was estimated by an average of pressures measured at 12 noon and 2 PM, and IOP after acetazolamide administration by an average of pressures measured at 4 PM and 6 PM. After a 1-week washout interval, the second experiment was carried out according to the same schedule of fluorophotometric and IOP measurements, acetazolamide administration, and fluorescein instillation, except that 20 \(\mu\)l 0.25% nipradilol was instilled in one randomly chosen eye and the vehicle in the fellow eye.

The effect on the uveoscleral outflow determined by the fluorophotometric method depends on the assumption that topical nipradilol has no significant effect on \(P_{ev}\). Therefore, effect on the uveoscleral outflow was also studied, by the anterior chamber perfusion method of Suguro et al.\(^{24}\) in generally anesthetized rabbits. At 12 noon, 10 mg/kg indomethacin was injected intraperitoneally, after IOP was measured in both eyes in animals under topical anesthesia. At 1 PM, 50 \(\mu\)l 0.25% nipradilol was instilled in one randomly chosen eye and the vehicle in the contralateral eye. After IOP measurement in both eyes at 1:30 PM, general anesthesia was induced with 25 mg/kg pentobarbital, and a 23-gauge needle was inserted into each eye and connected to a reservoir filled with 10\(^{-4}\) g/ml fluorescein isothiocyanate-dextran (MWt 50,700; Sigma) dissolved in artificial aqueous humor (BSS plus; Alcon). After removal of the aqueous humor through the needle, the solution was introduced into the anterior chamber and perfused for 30 minutes at a constant pressure of 15 mm Hg. After termination of perfusion, rabbits were killed by pentobarbital overdose and the eyes enucleated. After extensive washing with physiological saline, the cornea was excised and fluorescein isothiocyanate-dextran in the anterior chamber was removed as thoroughly as possible by washing with physiological saline. The eye was dissected into the anterior uvea, anterior sclera, posterior sclera, posterior uvea, and vitreous humor, discarding the lens. After centrifugation of the tissue homogenates, the fluorescein isothiocyanate-dextran concentration in the supernatant was determined fluorophotometrically, from which uveoscleral outflow was calculated.\(^{24}\)

**Effect on Optic Nerve Head Circulation**

The ONH circulation was measured by the laser speckle method, details of which have been described elsewhere.\(^{17,18}\) The apparatus consists of a fundus camera incorporating a diode laser (wavelength, 808 nm) and an image sensor.\(^{19}\) The laser beam was focused on the ONH and the scattered laser light was projected onto an image sensor corresponding to a field of 0.62 \(\times\) 0.62 mm in the rabbit fundus, on which the laser speckle pattern appears. The difference between the average speckle intensity and the speckle intensity for successive scanning of image speckles at pixels on the image sensor plane was calculated, and normalized blur value \((NB)\), the ratio of the average of the speckle intensity to this difference, was used as a quantitative index of speckle contrast that is indicative of blood velocity in tissue. One measurement took 0.18 seconds, and the average of \(NB\) level across any square area \((NB_{av})\) in the measurement field was calculated.\(^{18}\)

Intraocular pressure was measured at 12 noon under dim light, after the pupil was dilated with one drop of 0.4% tropicamide (Mydrin M; Santen Pharmaceutical, Osaka, Japan). Fifteen minutes after weak anesthesia was induced by intravenous injection of 15 mg/kg pental lunar valsal, the image speckles from a field of 0.42 \(\times\) 0.42 mm (70 \(\times\) 70 pixels in the image sensor plane) in the ONH free of visible surface vessels were recorded and the average \(NB\) level across this square field was calculated as quantitative index of ONH tissue blood velocity \((NB_{av,ONH})\). The average of six measurements taken at 1-minute intervals was adopted as the initial value. After the \(NB\) measurement, a fundus photograph was taken, to record the site of measurement. From the next day on, 20 \(\mu\)l 0.25% nipradilol was instilled in one randomly chosen eye of each animal and vehicle in the fellow eye twice daily at 11 AM and 11 PM for 14 days. During the treatment period, the light schedule was the same as described earlier, and the IOP was measured in both eyes at 12 noon on days 5 and 10 under dim light in animals under anesthesia of 15 mg/kg pentobarbital. On day 15, after the measurement of IOP at 12 noon, \(NB_{av,ONH}\) was measured again at the same site as described earlier.

**RESULTS**

**Effect of Topical Nipradilol on Intraocular Pressure**

Results obtained in the nipradilol-treated eye are summarized in Table 1 and those obtained in the vehicle-treated eye in Table 2. Topical nipradilol reduced the IOP in rabbits in a dose-dependent manner at concentrations between 0.06% and 0.25%. Maximum reduction of 6 mm Hg was measured at 2 hours. The effective duration with the 0.25% solution was 6 hours. A contralateral effect was evident at a concentration of 0.25% or higher.

During twice-daily application of 0.25% nipradilol for 14 days, nipradilol significantly \((P < 0.005)\) decreased IOP of the treated eye 3 hours after the morning dose (4.5 mm Hg, day 5; 4.8 mm Hg, day 10; and 4.4 mm Hg, day 14) compared with the baseline pressure (Fig. 2), although it significantly \((P < 0.05)\) reduced IOP in the vehicle-treated eye (1.6 mm Hg, 2.2 mm Hg, 2.1 mm Hg, respectively). There were no signs of tarsal or bulbar conjunctival hyperemia on biomicroscopic examination.

**Comparison of Nipradilol and Timolol**

Results are summarized in Table 3. Significant decreases (6 mm Hg and 4 mm Hg) were detected after a single instillation of 0.25% nipradilol and 0.5% timolol, respectively.
Nipradilol
Concentration of Nipradilol

A single, unilateral instillation of 0.25% nipradilol showed no significant effect on outflow to general blood circulation.

TABLE 2. Reduction in the Intraocular Pressure after Nipradilol Administration in Nipradilol-Treated Eye

<table>
<thead>
<tr>
<th>Concentration of Nipradilol</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06%</td>
<td>0.2 ± 0.6</td>
<td>0.7 ± 0.6</td>
<td>0.1 ± 0.8</td>
<td>0.3 ± 0.9</td>
<td>-0.4 ± 0.6</td>
<td>-0.7 ± 0.9</td>
<td>-0.3 ± 1.0</td>
</tr>
<tr>
<td>0.125%</td>
<td>2.1 ± 0.4*</td>
<td>3.4 ± 0.4†</td>
<td>1.9 ± 0.9*</td>
<td>1.1 ± 1.0</td>
<td>1.1 ± 0.8</td>
<td>0.5 ± 1.3</td>
<td>0.4 ± 1.3</td>
</tr>
<tr>
<td>0.25%</td>
<td>4.6 ± 0.6††</td>
<td>6.0 ± 0.6††</td>
<td>4.9 ± 0.7††</td>
<td>3.8 ± 1.1††</td>
<td>2.1 ± 0.8</td>
<td>1.4 ± 1.1</td>
<td>0.8 ± 1.1</td>
</tr>
<tr>
<td>0.5%</td>
<td>4.4 ± 0.5††</td>
<td>5.3 ± 0.6††</td>
<td>4.3 ± 0.9††</td>
<td>2.8 ± 1.2*</td>
<td>1.7 ± 1.0</td>
<td>0.8 ± 1.3</td>
<td>1.1 ± 1.3</td>
</tr>
</tbody>
</table>

Differences obtained in the right eye when treated with nipradilol solution and when treated with vehicle. Figures are mean ± SE (mm Hg) in 10 eyes of 10 animals.

1. Compared with 0.06%: *P < 0.05; †P < 0.01 (Sheffe’s F).
2. Compared with 0.125%: †P < 0.05; ‡P < 0.01 (Sheffe’s F).

At 2 hours, a significant difference in change in IOP was recorded between timolol and nipradilol. Addition of 0.5% timolol in combination with 0.25% nipradilol led to a significant further decrease in IOP at 1, 2, 3, and 4 hours, but no significant difference was observed after further addition of 0.5% timolol to 0.5% timolol.

Effect on Blood–Aqueous Barrier Permeability

Results are summarized in Table 4. A single instillation of 0.25% nipradilol showed no significant effects on anterior chamber fluorescein concentration.

Effect on Aqueous Flow Rate

The time course of the change in IOP after instillation of topical 0.25% nipradilol was administrated in the first experiment was similar to that obtained in the IOP experiment. After 2 hours, IOP was reduced by 6 mm Hg in nipradilol-treated eyes and by 2.6 mm Hg in vehicle-treated eyes compared, with the preinstillation level. When aqueous flow rate was determined fluorophotometrically, similar IOP reduction was also seen. After a single, unilateral instillation of 0.25% nipradilol, the aqueous flow rate was significantly reduced (an average of 17%) in treated eyes, and it was also significantly reduced (an average of 10%) in contralateral eyes (Table 5).

Effect on Outflow to General Blood Circulation and Uveoscleral Outflow

A single, unilateral instillation of 0.25% nipradilol showed no significant effect on the fluorophotometrically determined outflow facility to general blood circulation (C respect to the anterior chamber perfusion method also showed a significant increase of approximately 32%, compared with that in the contralateral control eye after a unilateral single instillation of 0.25% nipradilol (0.22 ± 0.01 versus 0.16 ± 0.01 µl/min, mean ± SE; n = 6; P = 0.0489 with paired t test).

Effect on Optic Nerve Head Microcirculation

Results are summarized in Table 7. Intraocular pressure measured 1 hour after the morning administration was reduced by approximately 4 mm Hg during the experimental period in the treated eye. The NBH Anglox in the treated eye showed a significant increase (approximately 10%) after 15 days of twice-daily instillation of 0.25% nipradilol, whereas it showed no significant change in the vehicle-treated eye.

DISCUSSION

Topical nipradilol reduced the IOP in rabbits in a dose-dependent manner with a maximum effect at 2 hours. Application of nipradilol at concentrations of 0.125% or higher led to reduction of IOP in the contralateral eye as well. The hypotensive effect of topical nipradilol at concentrations of 0.25% or higher was significantly greater than that of a concentration of 0.125%, but there was no significant difference in results between the 0.25% and 0.5% solutions. Thus, 0.25% nipradilol, which is the lowest concentration showing a close-to-maximum effect was used in the following experiments. During twice-daily, 14-day instillation of 0.25% nipradilol, no evidence of attenuation of the effect was found.

TABLE 2. Reduction in the Intraocular Pressure after Nipradilol Administration in Vehicle-Treated Eyes

<table>
<thead>
<tr>
<th>Concentration of Nipradilol</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.06%</td>
<td>0.2 ± 0.6</td>
<td>0.0 ± 0.8</td>
<td>0.3 ± 0.9</td>
<td>0.6 ± 0.8</td>
<td>1.1 ± 0.8</td>
<td>1.2 ± 0.6</td>
<td>-0.5 ± 0.8</td>
</tr>
<tr>
<td>0.125%</td>
<td>0.6 ± 0.6</td>
<td>1.4 ± 0.9</td>
<td>0.7 ± 0.9</td>
<td>0.6 ± 0.7</td>
<td>0.6 ± 0.7</td>
<td>0.5 ± 0.5</td>
<td>-0.3 ± 0.7</td>
</tr>
<tr>
<td>0.25%</td>
<td>2.1 ± 0.8*</td>
<td>2.6 ± 0.5†</td>
<td>2.2 ± 0.5†</td>
<td>2.0 ± 0.9</td>
<td>1.8 ± 0.9</td>
<td>2.0 ± 0.9</td>
<td>0.2 ± 0.8</td>
</tr>
<tr>
<td>0.5%</td>
<td>2.0 ± 0.6††</td>
<td>2.8 ± 0.7††</td>
<td>1.7 ± 0.7</td>
<td>2.1 ± 1.0</td>
<td>1.5 ± 1.0</td>
<td>1.5 ± 0.7</td>
<td>-0.2 ± 1.0</td>
</tr>
</tbody>
</table>

Differences obtained in the right eye when treated with nipradilol solution and when treated with vehicle. Figures are mean ± SE (mm Hg) in 10 eyes of 10 animals.

1. Compared with 0.06%: *P < 0.05; †P < 0.01.
2. Compared with 0.125%: †P < 0.05.
hypotensive effect, the instillation of 0.25% nipradilol 5 min-
utes after the first 0.5% timolol instillation, did not increase timolol’s
ocular hypotensive effect in monkeys and in rabbits. 13 14

Results of previous studies suggest that nitroglycerin also has
systemic β-blocking activity in humans as well. In the in vitro
system, however, β-blocking activity of nipradilol is approxi-
mately twice that of propranolol, 3 whereas that of timolol is
approximately seven times that of propranolol. 27 The maxi-
imum plasma concentration of nipradilol in rabbits was 0.049
µg/ml, 1 hour after a single instillation of 0.25% nipradilol, 25 26
whereas that of timolol was reportedly 0.188 µg/ml, 30 min-
utes after a single instillation of 0.5% timolol.28 Thus, when the

Table 3. Comparison of Nipradilol and Timolol

<table>
<thead>
<tr>
<th></th>
<th>Time after Instillation (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Timolol alone</td>
<td>4.4 ± 0.7</td>
</tr>
<tr>
<td>Timolol-added timolol</td>
<td>4.1 ± 0.4</td>
</tr>
<tr>
<td>Nipradilol alone</td>
<td>5.2 ± 0.6</td>
</tr>
<tr>
<td>Nipradilol-added timolol</td>
<td>5.8 ± 0.6‡†</td>
</tr>
</tbody>
</table>

*Difference obtained in the right eye between when treated with nipradilol solution and when treated with vehicle is shown. Figures are
mean ± SE (mm Hg) in 10 eyes of 10 animals.

†Compared with timolol alone: *P < 0.01, †P < 0.05 (Sheffe’s F).

‡Compared with timolol added timolol: #P < 0.01, $P < 0.05 (Sheffe’s F).
TABLE 5. Aqueous Flow Rate (Microliters/Minute)

<table>
<thead>
<tr>
<th></th>
<th>Nipradilol-Treated Eye</th>
<th>Vehicle-Treated Eye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control experiment</td>
<td>2.96 ± 0.11</td>
<td>2.93 ± 0.11</td>
</tr>
<tr>
<td>Nipradilol experiment</td>
<td>2.47 ± 0.10*</td>
<td>2.67 ± 0.09†</td>
</tr>
</tbody>
</table>

Figures indicate mean ± SE in eight animals. *P = 0.0064, compared with values in the control experiment (paired t-test). †P = 0.0355, compared with values in the control experiment (paired t-test).

0.25% concentration is used, systemic β-blocking activity of nipradilol after its topical instillation is expected to be considerably less than that after instillation of topical 0.5% timolol.

The average reduction in the outflow pressure (IOP – Pcv) in the 0.25% nipradilol-treated eye was approximately 35% between 11 AM and 3 PM. This value is considerably higher than the 17% reduction in IOP, suggesting that topical nipradilol has an effect not only on aqueous production, but also on the aqueous outflow system. Results of noninvasive fluorophotometric examination suggested that topical nipradilol had no significant effect on the outflow facility to the general blood circulation but induced a significant increase in uveoscleral outflow. Although the baseline uveoscleral outflow determined fluorophotometrically was considerably less than that determined by the anterior chamber perfusion method, the uveoscleral outflow-increasing effect of topical nipradilol was demonstrated by the two different methods, a noninvasive fluorophotometric method using conscious rabbits and an invasive method using anesthetized rabbits. The discrepancy in the baseline value of uveoscleral outflow may be partly attributed to effects of invasive and nonphysiological treatment in the anterior chamber perfusion method, general anesthesia, or both. Furthermore, Pcv was assumed in determining the uveoscleral outflow fluorophotometrically. Deviation of the actual Pcv value from the assumed value would have resulted in over- or underestimation of the uveoscleral outflow, which may also be partly responsible for the discrepancy.

Several selective α-blockers have been studied to determine their effects on aqueous humor dynamics in experimental animals and in human eyes. Serle et al. have reported that corynanthine does not change aqueous flow rate or outflow facility in rabbit eyes and suggest that corynanthine reduces IOP by increasing uveoscleral outflow. Kageyama et al. have reported that bunazosin, a highly selective α-blocker, lowers IOP by increasing uveoscleral outflow in rabbits. Oshihoka et al. have reported that bunazosin significantly reduces IOP in normal human eyes, probably by increasing uveoscleral outflow, without significant change in aqueous flow rate, tonographic outflow facility, or Pcv. Amosulalol, which blocks α1- and β-adrenoceptors is reported to reduce IOP by inhibiting aqueous production and by increasing uveoscleral outflow. Thus, the uveoscleral outflow-increasing effect of nipradilol may be at least partly attributed to its α1-blocking activity. In vitro, the α-blocking activity of topical nipradilol is approximately 1/6 that of prazosin and 1/50 that of bunazosin. Effective concentrations of topical prazosin and bunazosin, which reduce IOP in rabbits, are 0.0001% or higher and 0.005% or higher, respectively. Therefore α-blocking activity of topical 0.25% nipradilol should be sufficient to exert its pharmacologic effects in the eye. The nitroglycerin-like activity of nipradilol is approximately one fifth that of nitroglycerin. Effective concentrations of topical nitroglycerin to reduce IOP in rabbits are 0.03% or higher. The nitroglycerin-like activity of nipradilol may also be at least partly responsible for the IOP-reducing effect presently observed. Although care must be taken in extrapolating the results obtained in rabbits to humans, the effects of topical nipradilol on uveoscleral outflow would be advantageous, in that this drug may reduce IOP in eyes in cases in which the efficacy of β-blockers is insufficient.

In the present study, 15-day, twice-daily instillation of 0.25% nipradilol significantly increased the Nb aw/Nb ONH in the treated eye only, which agreed with our preliminary results obtained in fewer rabbits. The effective depth of sampling of the present instrument using diode laser is thought to be somewhat greater than 1 mm in the ONH tissue. Therefore, circulation in the prescleral and scleral regions would mainly contribute to the present Nb aw/Nb ONH, with some probable contribution from the retrolсорal region. Sugiya et al. have altered the ONH blood flow by 10% CO2 inhalation or by intravenous injection of a small amount of endothelin-1 (10^-10 mol/kg) and simultaneously measured the Nb aw/Nb ONH and the ONH blood flow by means of the H2-clearance method in the same rabbit ONH. Their findings show that CO2 inhalation significantly increases the Nb aw/Nb ONH (31%) and the ONH blood flow determined by the H2-clearance method (22%, on average), whereas intravenous injection of endothelin-1 significantly reduces the Nb aw/Nb ONH (22%) and the ONH blood flow.

TABLE 6. Effect of Topical Nipradilol on Outflow Facility to General and Uveoscleral Outflow

<table>
<thead>
<tr>
<th></th>
<th>Nipradilol Treated (control)</th>
<th>Vehicle Treated (control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outflow facility (Cgen) (microliters/min/mm Hg)</td>
<td>0.21 ± 0.003 (0.19 ± 0.01)</td>
<td>0.19 ± 0.01 (0.19 ± 0.01)</td>
</tr>
<tr>
<td>Uveoscleral outflow (Fv) (microliters/min)</td>
<td>0.28 ± 0.04* (0.08 ± 0.02)</td>
<td>0.10 ± 0.02 (0.05 ± 0.03)</td>
</tr>
</tbody>
</table>

Figures in parenthesis indicate the result obtained in the control experiment (mean ± SE, n = 8). *P = 0.006, compared with values in the control experiment (paired t-test). †P = 0.006, compared with values in the vehicle-treated eye (paired t-test).
determined by the H₂-clearance method (19%, on average). Furthermore, relative changes in the \(NB_{\text{avCONH}}\) and the ONH blood flow determined by the H₂-clearance method show a high correlation (\(r = 0.92\)). Although \(NB_{\text{avCONH}}\) is primarily a quantitative index of tissue blood velocity, the results of Sugiyama et al. indicate that \(NB_{\text{avCONH}}\) can also be used as an index of the blood flow through the ONH tissue.

The present results showed that a 15-day, twice-daily regimen of 0.25% nipradilol had a beneficial effect on ONH circulation. Because \(\beta\)-blocking activity itself is related more to peripheral vasoconstriction than to vasodilation, the effect presently observed is thought to be related to pharmacologic effects of nipradilol other than its \(\beta\)-blocking activity. After 7-day, once-daily instillation of 0.25% nipradilol, its plasma concentration is reported to be approximately 0.01 to 0.02 \(\mu M\). Results of studies in vitro indicate, that nipradilol’s vasodilating effect, which is attributable to its \(\alpha\)-blocking and nitroglycerin-like activities, becomes evident at concentrations of 0.1 \(\mu M\) or higher. Thus, it is unlikely that the presently observed effect on the ipsilateral \(NB_{\text{avCONH}}\) is attributable to systemically absorbed nipradilol. The lack of effect in the contralateral ONH also supports this interpretation. After a single instillation of 1% nipradilol, its concentration in the posterior sclera or retina-choroid is reported to reach a concentration level of 0.5 to 1 \(\mu M\) a figure that agrees with those reported for other \(\beta\)-blockers, such as timolol, betaxolol, or carteolol. These experimental results suggest that the increase in the \(NB_{\text{avCONH}}\) presently observed only in the ipsilateral ONH after 15-day, twice-daily 0.25% nipradilol instillation may be at least partly attributable to the pharmacologic activities of nipradilol that penetrated locally, possibly by the pericentral route. Any or all pharmacologic properties in nipradilol—those of \(\beta\)-receptor blockade, \(\alpha\)-receptor antagonist, and nitric oxide donor—could contribute to improved ONH blood flow. However, it must be noted that the present experiment was not designed to elucidate the pharmacologic mechanism of nipradilol’s \(NB_{\text{avCONH}}\) increasing effect. Elucidation of this problem awaits future studies.

An increase in the ocular perfusion pressure (OPP) induced by the IOP decrease is another possible factor contributing to the present finding. Although OPP could not be calculated in the present experiment because blood pressure was unknown, the difference in IOP between the nipradilol- and the vehicle-treated eyes should be the same as the difference in OPP. If the incremental increase in OPP after instillation is the main cause of the \(NB_{\text{avCONH}}\) increment, a correlation between bilateral difference in IOP, that is, OPP, and that in \(NB_{\text{avCONH}}\) after 15 days’ instillation of nipradilol is to be expected. No significant correlation between them was observed, before or after the 15-day treatment. Furthermore, the difference in IOP between the nipradilol-treated eyes and the vehicle-treated eyes was approximately 3 mm Hg after the 15-day treatment, whereas OPP was approximately 70 to 80 mm Hg in normal rabbits, suggesting that the difference in the OPP between the bilateral eyes would be only 4%. These results suggest that the increase in OPP induced by reduction of IOP would not be the main cause of the \(NB_{\text{avCONH}}\) increment. Using the same instrument and experimental design, we previously studied the effect of topical bunazosin, a highly selective \(\alpha\)-blocker, on the \(NB_{\text{avCONH}}\) in rabbits. Although 3-week, twice-daily instillation of 0.01% bunazosin reduces the IOP by approximately 3 mm Hg only in the ipsilateral bunazosin-treated eye, it causes no significant effect on the \(NB_{\text{avCONH}}\) of the ipsilateral eye. Because bunazosin should not exert a vasoconstrictive effect, this result may also favor the explanation made earlier.

In summary, topical nipradilol decreased the IOP in rabbit eyes by reducing the aqueous flow and by increasing uveoscleral outflow. Furthermore, topical nipradilol had beneficial effects on ONH circulation. Topical nipradilol may have an advantage, in comparison with presently used \(\beta\)-blockers, as an antiglaucoma agent, considering its effect on uveoscleral outflow and possible beneficial effect on ONH circulation. The potential of topical nipradilol observed in this study in rabbit eyes deserves further investigation.

References