Effects of Nilvadipine, a Calcium Antagonist, on Rabbit Ocular Circulation and Optic Nerve Head Circulation in NTG Subjects

Ken Tomita,1 Makoto Araie,2 Yasuhiro Tamaki,3 Miyuki Nagahara,2 and Tetsuya Sugiyama4

PURPOSE. To study the effects of nilvadipine, a Ca2+ antagonist, on tissue circulation in the optic nerve head (ONH), choroid, and retina in rabbits and on the ONH circulation in normal tension glaucoma (NTG) patients.

METHODS. Nilvadipine (3.2 µg/kg) or vehicle solution was injected intravenously into urethane-anesthetized rabbits, and the normalized blur value (NB), a quantitative index of in vivo tissue blood velocity, was measured in the choroid and in an area of the ONH and retina free of visible surface vessels before and for 90 minutes after injection, using the laser speckle method. The effects of nilvadipine on the ONH circulation was also studied using the H2 gas clearance method in separate groups of rabbits. Oral nilvadipine (4 mg/d) or placebo was administered to NTG patients in a double-masked manner, and NB in an area of the ONH rim free of visible surface vessels was measured by the same method before and 2, 4, 8, and 12 weeks after administration.

RESULTS. The NB obtained from the ONH, choroid, or retina during the experimental period was increased by approximately 10% to 25% in the nilvadipine group compared with the NB in the control group (P < 0.0001, ANOVA), although systemic condition parameters and intraocular pressure (IOP) showed no significant intergroup difference except for a transient decrease in blood pressure in the nilvadipine groups. Blood flow rate in the ONH determined by the H2 gas clearance method also showed an approximately 25% increase in the nilvadipine group. The NB in the ONH of the oral nilvadipine-treated patients was significantly increased, by approximately 20% compared with the placebo-treated patients throughout the follow-up period. No significant intergroup difference was seen in blood pressure, pulse rate, or IOP.

CONCLUSIONS. Nilvadipine increased blood velocity and, probably, blood flow in the ONH, choroid, and retina of rabbits. It also increased blood velocity in the ONH of NTG patients. (Invest Ophthalmol Vis Sci. 1999;40:1144-1151)

Previous studies have suggested that not only the mechanical damage due to intraocular pressure (IOP) but also compromise of the tissue circulation in the optic nerve head (ONH) play a significant role in the development of glaucomatous damage in the ONH.1,2 Calcium antagonists, which have been widely used for treatment of systemic hypertension, reduce blood vessel tone by inhibiting the entry of calcium ion intracellularly, thus causing relaxation of vascular smooth muscle cells and increased regional blood flow in several organs.3-5 Some of the calcium antagonists have been suggested to retard the progression of visual field damage in a subset of normal tension glaucoma (NTG) patients,6-9 which may be at least partly attributed to their vasodilating effect on tissues suffering from glaucomatous damage.6,9 In fact, intravenous (IV) injection of nicardipine, one of the dihydropyridine calcium antagonists,10 was reported to increase the ONH blood flow as measured by laser Doppler flowmetry in anesthetized cats at a dose of 20 µg/kg or 100 µg/kg.11 There may be some advantages in using nilvadipine, another dihydropyridine calcium antagonist used in Japan as a systemic hypotensive drug since 1989,12-14 as a potential vasodilator of ocular neural tissues. (1) Nilvadipine increased vertebral blood flow more effectively than nifedipine or nicardipine in anesthetized dogs.12 (2) Oral nilvadipine was reported to increase the regional blood flow, including cerebral blood flow, more effectively than oral nifedipine in elderly hypertensive patients, and this effect of oral nilvadipine was evident at a dose of 2 mg/d, which shows little effect on systemic blood pressure.15 (3) In subjects without hypertension, systemic blood pressure is affected little by oral nilvadipine.16 In an attempt to investigate the potential of nilvadipine as a vasodilator of ocular neural tissues, we first studied the effects of nilvadipine on tissue circulation in the retina, ONH,

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and choroid in rabbits using the noninvasive laser speckle method.\textsuperscript{17-19} Second, ONH blood flow was determined in the nilvadipine-treated rabbits by the hydrogen gas clearance method to confirm the results obtained by the laser speckle method. Finally, the effects of oral nilvadipine on the ONH were studied in a group of NTG patients.

\section*{MATERIALS AND METHODS}

\subsection*{Laser Speckle Method}

Tissue circulation in the ONH, choroid, and retina was evaluated using the laser speckle method, details of which have been described previously\textsuperscript{17-19} and are briefly described below. An apparatus consisting of a fundus camera equipped with a diode laser (wavelength of 808 nm) was used to measure ONH and choroid circulation in the rabbit and ONH circulation in NTG patients.\textsuperscript{16,18} Another apparatus equipped with an argon laser with its blue component (wavelength of 488 nm) was used to measure retinal circulation in the rabbit.\textsuperscript{17}

The fundus where the laser beam was focused was observed by means of an infrared charge-coupled device (CCD) camera. The scattered light was imaged on an image sensor of 100 $\times$ 100 pixels, corresponding to a field of 0.62 $\times$ 0.62 mm in the rabbit fundus and 1.06 $\times$ 1.06 mm in the human fundus, where the speckle pattern appeared. The average of the speckle intensity and the speckle intensity for successive scans of the image speckles at the pixels on the sensor plane was calculated, and the ratio of the average of the speckle intensity to this difference was defined as Normalized Blur (NB). NB is nearly equivalent to the reciprocal of speckle contrast given by Fercher and Briers\textsuperscript{20} and is primarily influenced by the number of blood cells in a sampling volume.\textsuperscript{21} The results were displayed in color graphics showing the two-dimensional variation of the NB level over the field of measurement. The average of the NB level in any square or rectangular area in the measurement field was calculated as NB$_{av}$.

\subsection*{Drugs Used}

For animal experiments, nilvadipine (5-isopropyl 3-methyl 2-cyano-6-methyl-1(3-nitrophenol)-1,4-dihydro-3,5-pyridinedi-carboxylate) was supplied by Fujisawa Pharmaceutical (Tokyo, Japan). It was dissolved in a mixture of EtOH, polyethylene glycol 400, and distilled water (2:1:7) and diluted with physiological saline before use and then passed through a filter (pore size, 0.2 $\mu$m; Millipore, Bedford, MA) to remove possible contaminants. In patients, oral nilvadipine (Nivadil; Fujisawa) or a placebo tablet was given twice daily at a dose of 4 mg/d (2 tablets/d).

\subsection*{Animal Experiment}

\subsubsection*{Normalized Blur Measurements in ONH or Choroid}

Japanese albino rabbits weighing 2.9 kg to 3.1 kg were used and handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. General anesthesia was induced by IV injection of 0.9 g/kg to 1.1 g/kg urethane. After induction of general anesthesia, the femoral artery was cannulated with a polyethylene catheter connected to a pressure transducer to measure the femoral arterial blood pressure and pulse rate, and the animal was put in a modified restraining box equipped with a heat pad. Arterial PO$_2$, PCO$_2$, and pH were checked before and at 30, 60, and 90 minutes after the injection of nilvadipine or vehicle solution, using a pH/blood gas analyzer (model 170; Corning Glass, Corning, NY), and body temperature was monitored with a rectal thermometer. In both eyes, the pupil was dilated with one drop of 0.4% tropicamide (Mydrin M, Santen Pharmaceutical, Osaka, Japan).

For measurement of ONH tissue blood velocity, the average of NB levels (NB$_{av}$) was recorded over a square area of 0.42 $\times$ 0.42 mm ($70 \times 70$ pixels in the sensor plane) in the ONH area, where no discrete surface vessels were visible.\textsuperscript{18} For measurement of choroidal tissue blood velocity, NB$_{av}$ was recorded over a square area of 0.62 $\times$ 0.62 mm (100 $\times$ 100 pixels in the sensor plane), approximately one papillary diameter below the ONH and outside the medullary area.\textsuperscript{18} Both measurements were made in one randomly chosen eye in separate groups of animals. Measurement of the NB$_{av}$ was carried out every 0.125 second over 1 second\textsuperscript{12} during which no eye movement was encountered, using the apparatus equipped with diode laser.\textsuperscript{12} The results obtained during the 1-second measurement period were further averaged, and this obtained value will be referred to as ONH-NB$_{av}$ or choroid-NB$_{av}$. Eye movement during the measurement was checked by the method previously described.\textsuperscript{17,18} Nilvadipine solution was injected at a dose of 3.2 $\mu$g/kg IV (0.2 ml solution/kg) into one group of rabbits (ONH or choroid-nilvadipine group) and the same volume of the vehicle solution, using a pH/blood gas analyzer (model RBF-222; Biomedical Science, Kanazawa, Japan), the capillary blood flow in the ONH was influenced by the number of blood cells in a sampling volume.\textsuperscript{21} The results were displayed in color graphics showing the two-dimensional variation of the NB level over the field of measurement. The average of the NB level in any square or rectangular area in the measurement field was calculated as NB$_{av}$.

\subsubsection*{Normalized Blur Measurements in Retinas}

Japanese albino rabbits weighing 1.9 kg to 2.5 kg were used because in this species the medullary area containing retinal blood vasculature can be easily identified. After mydriasis in both eyes, the NB$_{av}$ over a square retinal area of 0.62 $\times$ 0.62 mm (100 $\times$ 100 pixels in the sensor plane) free of visible surface vessels and approximately one papillary diameter away from the ONH along the medullary rays were recorded in one randomly chosen eye, using the apparatus equipped with argon laser.\textsuperscript{17} With this apparatus, one measurement took 0.18 second, and the average of three measurements was adopted as retina-NB$_{av}$. General anesthesia and monitoring of systemic condition parameters were carried out as described above. After nilvadipine or the vehicle solution injection, the retina-NB$_{av}$ and IOP measurements were carried out in the nilvadipine and control groups in the same manner as above.

\subsection*{Measurements by Hydrogen Gas Clearance Method}

Japanese albino rabbits weighing 2.7 kg to 3.2 kg were anesthetized as described above. Under observation with a vitreotomy lens, a hydrogen electrode (platinum needle with a 0.3 mm diameter Pt/Ir tip) was inserted through the vitreous body from the pars plana into a lower portion of the ONH to a depth of approximately 0.7 mm. A reference electrode was fixed in the subcutaneous tissue of the animal head. Using a hydrogen gas clearance flowmeter (model RBF-222; Biomedical Science, Kanazawa, Japan), the capillary blood flow in the ONH was...
calculated on the basis of the hydrogen density half-life after inhalation of 4% hydrogen gas by mask at a rate of 5.0 l/min for 4 minutes. Calculations of blood flow rate were made before and every 15 minutes for 90 minutes after the drug injection. Nilvadipine or vehicle solution was given at the same dosage and in the same manner as in the laser speckle experiment, and the blood pressure and pulse rate were monitored every 15 minutes.

**Human Study**

The effects of oral nilvadipine on the ONH circulation was studied in a small group of patients in a double-masked, placebo-controlled manner. Twelve NTG patients (8 women and 4 men) with mild to moderate visual field damage and without other ocular diseases were included in this study (Table 1). They averaged 56 years of age and had no history of taking systemic drugs or contraindication to systemic calcium antagonist therapy, and they had no other systemic diseases such as hypertension or hypotension. Topical ocular hypotensive medication, if any, had been discontinued at least 1 month before the study. After measuring the IOP in both eyes and brachial arterial blood pressure, both pupils were dilated with one drop of Mydriatic M, and the NBav was recorded from an area of approximately 0.1 X 0.1 to 0.2 X 0.2 mm (10 X 10 to 20 X 20 pixels in human eyes) in the neuroretinal rim of the temporal half of the ONH free of visible surface vessels, using the apparatus equipped with diode laser. Normalized blur measurements were carried out in both eyes and repeated every 0.125 second over 5 seconds. Because NB fluctuations are synchronous with heartbeats, NB measurements were averaged over three heart beats, during which there was no eye movement, to obtain ONH-NBav; a polaroid fundus photograph was taken to record the site of measurement. Eye movement during measurement was checked by the method previously described and by inspecting successive measurements taken at 0.125-second intervals displayed in color graphics, using the visible surface vessels in the measurement field as markers. The patients were randomly assigned to either the nilvadipine or placebo group. The former received oral nilvadipine (Nilvadil) 4 mg/d (2 mg X 2 times/d), and the latter received two tablets of placebo twice daily. Patients were asked to take tablets at 8:00 AM and 10:00 PM. During the follow-up period of 12 weeks, patients were examined 2, 4, 8, and 12 weeks after entry into the study; at each visit, compliance was confirmed by interview and the IOP, brachial arterial blood pressure, and ONH-NBav were measured as described previously.

The ONH-NBav was measured in the same site as at 0 weeks, using surface vessels as markers. All measurements were carried out between 11:00 AM and 1:00 PM by an investigator unaware of the treatment. The visual field was examined using the central 30-2 program of the Humphrey Field Analyzer (Humphrey Instruments, San Leandro, CA) within 1 month before and after the study period of 3 months. The protocol of the study was approved by the Ethical Review Committee of the University of Tokyo School of Medicine, and informed consent was obtained from each participant after the procedure had been fully explained.

**RESULTS**

**Animal Experiment**

Rabbits in which systemic condition parameters immediately before the drug or vehicle injection were outside the normal range were not included in the study, and animals in which any of the systemic condition parameters showed abnormal change during the experiment were also eliminated from the study. According to the above criteria, approximately 1 of 10 of the animals must be discarded, which is probably attributable to physical weakness of a part of the animals used.

**Normalized Blur Measurements in ONH or Choroid.**

In both the ONH and choroid experiments, no significant difference was seen between the nilvadipine and control groups in IOP or any of the systemic condition parameters except for blood pressure, before and during the experiment.

In the nilvadipine group, the mean femoral arterial blood pressure significantly decreased from its baseline for 5 minutes after the injection; it showed no significant difference from its baseline at 10 minutes postinjection or later. The blood pressure in the control group tended to decrease but to be not statistically significantly (Figs. 1, 2). The baseline ONH-NBav showed no significant intergroup difference, averaging 12.3 ± 0.9 (mean ± SE, n = 10) and 13.3 ± 0.7 in the ONH-nilvadipine and ONH-control group, respectively. The time change in the ONH-NBav after the drug or vehicle solution injection was compared between the nilvadipine and the control groups based on the difference in the ONH-NBav from the baseline (∆ONH-NBav; Fig. 1). ∆ONH-NBav was significantly greater than zero at 4 minutes or later in the nilvadipine group; ∆ONH-NBav did not vary significantly from zero in the control group throughout the experimental period. The mean measurements of ∆ONH-NBav at different time points were significantly dif-

### Table 1. Baseline Patient Data

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Nilvadipine (4 mg/d)</th>
<th>Placebo</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients (F/M)</td>
<td>6 (3/3)</td>
<td>6 (2/1)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>53 ± 15</td>
<td>58 ± 13</td>
<td></td>
</tr>
<tr>
<td>Mean deviation (dB)*</td>
<td>−9.3 ± 6.4</td>
<td>−7.7 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>IOP (mm Hg)</td>
<td>13.5 ± 2.4</td>
<td>14.7 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)†</td>
<td>92 ± 1.4</td>
<td>95 ± 17</td>
<td></td>
</tr>
<tr>
<td>Ocular perfusion pressure (mm Hg)‡</td>
<td>50 ± 10</td>
<td>47 ± 10</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD. Mean deviation and intraocular pressure were averaged in 12 eyes of 6 patients. No significant intergroup difference is seen in any of the parameters.

* The 30-2 program of Humphrey Visual Field Analyzer.
† Diastolic blood pressure + (mean blood pressure − diastolic blood pressure).
‡ (%) (mean blood pressure − intraocular pressure).
Effects of Nilvadipine on Ocular Circulation

FIGURE 1. Time course of the difference in ONH-NB\textsubscript{av} before and after nilvadipine (3.2 \(\mu\)g/kg) or vehicle injection (ONH-NB\textsubscript{av}) and mean blood pressure in nilvadipine (●) and vehicle-treated (○) rabbits. Each plot represents mean and error bar SE in 10 rabbits. Intergroup difference in ΔONH-NB\textsubscript{av} was significant on ANOVA for data obtained by sequential measurements (group, \(P < 0.0001\); group \(\times\) time, \(P < 0.0001\)).

The baseline choroid-NB\textsubscript{av} showed no significant intergroup difference, averaging 26.0 ± 2.9 (mean ± SE, \(n = 10\)) and 26.8 ± 0.9 in the choroid-nilvadipine and choroid-control groups, respectively. The time course of the difference between the choroid-NB\textsubscript{av} and the baseline (Δchoroid-NB\textsubscript{av}) is shown in Figure 2. Δchoroid-NB\textsubscript{av} was significantly greater than zero at 10 minutes or later in the nilvadipine group; it did not significantly vary from zero throughout the experiment in the control group. The mean measurements of Δchoroid-NB\textsubscript{av}.

FIGURE 2. Time course of the difference in choroid-NB\textsubscript{av} before and after nilvadipine 3.2 \(\mu\)g/kg) or vehicle injection (Δchoroid-NB\textsubscript{av}) and mean blood pressure in nilvadipine (●) and vehicle-treated (○) rabbits. Each plot represents mean and error bar SE in 10 rabbits. Intergroup difference in Δchoroid-NB\textsubscript{av} was significant on ANOVA for data obtained by sequential measurements (group, \(P < 0.0001\); group \(\times\) time, \(P < 0.0001\)).
Normalized Blur Measurements in Retinas. No significant intergroup difference was seen in the systemic condition parameters before the injection. During the experiment, the pulse rate, body temperature, and arterial blood pH remained unchanged in both groups; IOP and Pco₂ decreased slightly, and Po₂ increased. However, no significant intergroup difference was seen in any of the parameters at any time of measurement (30, 60, and 90 minutes). In the nilvadipine group, the mean femoral arterial blood pressure significantly decreased from its baseline for 5 minutes after the injection; however, it showed no significant difference from its baseline at 10 minutes postinjection or later (Fig. 3). The baseline retina-NBav showed no significant intergroup difference, averaging 12.0 ± 0.8 and 10.8 ± 1.1 (mean ± SE, n = 10) in the nilvadipine and control groups, respectively. The time course of the difference between the retina-NBav and the baseline (Δretina-NBav) is shown in Figure 3. ARetina-NBav was significantly greater than zero at 35 minutes or later in the nilvadipine group; it was significantly smaller than zero at several points during the experiment in the control group. The mean measurements of Δretina-NBav at different time points were significantly different between the nilvadipine and control groups [ANOVA of repeated measurements, P < 0.0001 (group), P < 0.0001 (group × time)].

Measurements by Hydrogen Gas Clearance Method. The blood pressure and pulse rate, which were measured every 15 minutes, showed no significant change throughout the experiment (Table 2). The baseline ONH capillary blood flow averaged 33.4 ± 3.2 (mean ± SE, n = 6) and 38.8 ± 5.21 ml/min per 100 g tissue in the nilvadipine and control groups, respectively; no significant intergroup difference was seen. The time course of the difference between the ONH capillary blood flow and the baseline is shown in Table 2, and intergroup difference is significant [ANOVA of repeated measurements, P < 0.0001 (group), P < 0.0001 (group × time)].

**Table 2. Measurement of ONH Blood Flow by Hydrogen Gas Clearance Method**

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>15 Min</th>
<th>30 Min</th>
<th>45 Min</th>
<th>60 Min</th>
<th>75 Min</th>
<th>90 Min</th>
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</thead>
<tbody>
<tr>
<td>ΔONH-BF&lt;sub&gt;nilvadipine&lt;/sub&gt; (ml/min per 100 g)</td>
<td>0</td>
<td>0.7 ± 1.6</td>
<td>6.7 ± 1.2</td>
<td>7.4 ± 1.5</td>
<td>7.6 ± 2.5</td>
<td>9.6 ± 2.1</td>
<td>9.8 ± 2.1</td>
</tr>
<tr>
<td>ΔONH-BF&lt;sub&gt;control&lt;/sub&gt; (ml/min per 100 g)</td>
<td>0</td>
<td>-0.7 ± 2.7</td>
<td>-1.9 ± 2.2</td>
<td>0.8 ± 0.7</td>
<td>-0.9 ± 2.6</td>
<td>-0.6 ± 2.6</td>
<td>-0.3 ± 2.4</td>
</tr>
<tr>
<td>Mean BP&lt;sub&gt;nilvadipine&lt;/sub&gt; (mm Hg)</td>
<td>100 ± 4</td>
<td>96 ± 3</td>
<td>98 ± 3</td>
<td>98 ± 3</td>
<td>96 ± 3</td>
<td>99 ± 2</td>
<td>97 ± 2</td>
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<tr>
<td>Mean BP&lt;sub&gt;control&lt;/sub&gt; (mm Hg)</td>
<td>103 ± 3</td>
<td>101 ± 8</td>
<td>100 ± 4</td>
<td>101 ± 4</td>
<td>102 ± 4</td>
<td>103 ± 5</td>
<td>107 ± 4</td>
</tr>
</tbody>
</table>

Values are mean ± SE in 6 rabbits. ΔONH-BF<sub>nilvadipine</sub> (ΔONH-BF<sub>control</sub>) indicates difference in ONH blood flow between that before drug or vehicle injection and that at each time point in nilvadipine (control) group; mean BP<sub>nilvadipine</sub> (Mean BP<sub>control</sub>) indicates mean arterial blood pressure in nilvadipine (control) group. Intergroup difference is significant for ΔONH-BF (P < 0.0001, ANOVA) but not for mean BP.

**Figure 3.** Time course of the difference in retina-NBav before and after nilvadipine 3.2 μg/kg or vehicle injection (Δretina-NBav) and mean blood pressure in nilvadipine (○) and vehicle-treated (□) rabbits. Each plot represents mean and error bar SE in 10 rabbits. Intergroup difference in Δretina-NBav was significant on ANOVA for data obtained by sequential measurements (group, P < 0.0001; group × time, P < 0.0001).
peated measurements, $P < 0.0001$ (group), $P = 0.0001$ (group $\times$ time)).

**Human Study**

The average of NB and IOP measurements obtained from both eyes of a patient was adopted as the value representing that patient. In both the nilvadipine and placebo groups, the IOP and systemic, diastolic, and mean blood pressures showed no significant change from the baseline or intergroup difference at any of the measurement points (paired or unpaired $t$-test, $P > 0.1$). The ocular perfusion pressure, calculated as $[2/3 \times$ (mean blood pressure) $- IOP]$, showed no significant change from the baseline at any of the measurement points (paired $t$-test, $P > 0.1$) in both groups (Table 3).

The baseline ONH-NBav showed no significant intergroup difference, averaging $19.8 \pm 3.8$ and $17.2 \pm 4.3$ (mean $\pm$ SD, $n = 6$) in the control and nilvadipine groups, respectively. It showed significant increase from the baseline in the nilvadipine group; no significant change was seen in the control group (Table 3). Determination of the difference between the ONH-NBav and the baseline ($\Delta$ONH-NBav) showed a significant intergroup difference [ANOVA of repeated measurements, $P < 0.0001$ (group), $P = 0.0096$ (group $\times$ time)].

**DISCUSSION**

The present study first investigated effects of systemic nilvadipine, a calcium antagonist with less effect on the blood pressure in normotensive subjects, on ocular circulation in rabbits and NTG patients and demonstrated that this drug favorably affected the retina, ONH, and choroid circulation in rabbits and the ONH circulation in patients.

The NB is primarily a quantitative index of tissue blood velocity. However, it is also influenced by the number of blood cells in the sampling volume; in an experiment using glass capillary tubes with a diameter of 50 $\mu m$ to 300 $\mu m$ as analogues of blood vessels, it was found that the greater the diameter of a tube, the greater the NB. In rabbits wherein ocular blood flow rate was artificially changed by increasing the IOP, infusion of 10% CO$_2$, or by intravenous injection of a small amount of endothelin-1, NBav showed a good correlation with the blood flow rate determined by the microsphere method in the retina or choroid or by the hydrogen gas clearance method only indirectly estimates intraluminal blood cell movements, the similarity of nilvadipine’s effect on ONH tissue circulation determined by these two methods is thought to support not only nilvadipine’s beneficial effect on ONH tissue circulation but also the validity of the laser speckle method.

In the animals in the present experiment, the mean femoral arterial blood pressure reached the lowest level 1 minute after IV injection of 3.2 $\mu g/kg$ nilvadipine and remained significantly lower than that in the control group up to 10 minutes after injection. The blood flow in the ocular tissue can be simply expressed according to the following formula: Blood flow = ocular perfusion pressure (OPP)/R, where R is the vascular resistance and OPP is mean arterial blood pressure $- 14$ mm Hg $- IOP$. The lack of change in the IOP implies that the OPP directly depends on the mean arterial blood pressure. The retina-NBav showed some reduction during the OPP decrease phase, although it was not statistically significant. In the ONH and choroid, however, the NBav showed no concurrent reduction. The lack of reduction in the ONH-NBav during the OPP decrease phase may be explained by the relaxing effect of nilvadipine, a calcium antagonist, on vascular smooth muscle cells; (i.e., reduction in R) and/or by autoregulation of blood flow in the ONH and choroid. In our previous experiment, the ONH- and retina-NBav, but not choroid-NBav, showed autoregulation against an acute OPP decrease induced by IOP increase. Thus, a reduction in the retina-NBav and a lack of reduction in the ONH- and choroid-NBav during the OPP decrease phase suggest that the autoregulation mechanism is rather unlikely. One explanation for the discrepancy in nilvadipine effects between the ONH or choroid and the retina is as follows: In the choriocapillaries, there are numerous fenestrations that allow the nilvadipine in the vessel to rapidly reach

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>2 Wk</th>
<th>4 Wk</th>
<th>8 Wk</th>
<th>12 Wk</th>
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<tbody>
<tr>
<td>(\Delta)ONH-NBav (nilvadipine group)</td>
<td>0</td>
<td>2.2 ± 0.5</td>
<td>3.3 ± 1.2</td>
<td>3.8 ± 1.2</td>
<td>4.0 ± 1.3</td>
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<tr>
<td>(\Delta)ONH-NBav (control group)</td>
<td>0</td>
<td>-0.7 ± 0.5</td>
<td>-0.9 ± 0.4</td>
<td>-1.3 ± 0.6</td>
<td>-0.9 ± 0.4</td>
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<tr>
<td>OPP (mm Hg) (nilvadipine group)</td>
<td>50 ± 4</td>
<td>47 ± 3</td>
<td>47 ± 2</td>
<td>46 ± 2</td>
<td>46 ± 3</td>
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<tr>
<td>OPP (mm Hg) (control group)</td>
<td>47 ± 4</td>
<td>46 ± 4</td>
<td>48 ± 3</td>
<td>43 ± 4</td>
<td>45 ± 3</td>
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</table>

Values are means $\pm$ SE in 6 patients. \(\Delta\)ONH-NBav indicates difference in ONH-NBav between that before and at each time point of measurement. Intergroup difference is significant for \(\Delta\)ONH-NBav ($P = 0.0096$, ANOVA) but not for OPP. OPP, ocular perfusion pressure.
the smooth muscle cells in the outer wall vessels. Although the ONH vasculature has a blood-nerve barrier property,25 substances are known to diffuse into the ONH tissue from the peripapillary choroid.27 In contrast, there should be some lag time before the nilvadipine effect on the vascular smooth muscle cells becomes evident in retinal vessels, because nilvadipine must first penetrate the blood-retinal barrier to reach vascular smooth muscle cells.29 Furthermore, the effect of nilvadipine may differ somewhat among the tissues. Calcium antagonists are not general peripheral vasodilators, nor do they show a uniform pattern of preferential sites of action, and divergent effects of nifedipine have been reported in afferent and efferent arterioles in the rat kidney.29 Similar differences in the vascular bed selectivity may in part account for the above-mentioned difference between the retina-NBav and the ONH-or choroid-NBav during the OPP decrease phase.

After mean arterial blood pressure approached baseline level, the NBav in treated animals showed an increase compared with that in the control animals in all tissues examined. Although urethane anesthesia affects the cardiovascular system and vascular smooth muscle,30,31 the significant difference between the nilvadipine- and vehicle-treated control animals indicates that this effect is probably attributable to the vascular relaxing effect of nilvadipine. The nilvadipine effects on the ONH- and retina-NBav, presently obtained in urethane-anesthetized rabbits were different from the effects obtained with nicardipine, another calcium antagonist, in pentobarbital-anesthetized rabbits; IV nicardipine considerably increased the choroid NBav,24 but little affected the ONH- or retina-NBav.32 A more lipophilic chemical property of nilvadipine than that of nicardipine33 would be advantageous in reaching vascular smooth muscles through the blood-nerve barrier.

In human eyes, the coefficient of reproducibility of 24-hour interval measurements of the ONH-NBav was 13%.19 Oral nilvadipine at a dose of 4 mg/d was found to cause about a 20% increase in the ONH-NBav of the NTG patients participating in the present study. According to Koelle et al.,34 the penetration depth of infrared laser (wavelength 811 nm) in the cat optic nerve exceeds 1 mm. As found in the previous study23 and also in this rabbit experiment, the ONH-NBav showed a good correlation with the ONH blood flow rate determined by the hydrogen gas clearance method in which a hydrogen electrode was inserted into the ONH tissue to a depth of approximately 0.7 mm. Thus, the present increase in the ONH-NBav is thought to mainly reflect change in circulation in the prelaminar to laminar part of the ONH. The present finding is compatible with those of Netland et al., which suggested that topical verapamil increased capillary blood velocity in the ONH or decreased the resistive index of the central retinal artery in human subjects through its calcium channel blocking activity.35,36 Furthermore, the present effect of nilvadipine could be maintained at least for 3 months under treatment with daily oral dose of 4 mg.

The ocular vascular effect of nilvadipine has a potential clinical role in the treatment of open angle glaucoma, especially NTG or other ocular ischemic diseases. Compromise of the optic nerve circulation has been thought to be partly involved in the development of open angle glaucoma.1,2 Its contribution may be relatively more significant in NTG eyes in which clinical features are very similar to those of primary open angle glaucoma despite the recorded IOPs that are always in statistically normal range.37,38 According to a population-based glaucoma survey carried out in 8126 Japanese aged 40 years or older,39 the percentage rate of glaucomatous visual field damage shows an IOP-dependent increase starting at 15 mm Hg and a marked IOP-dependent increase occurring at a pressure of 21 mm Hg. On the other hand, a constant and non-IOP-dependent occurrence of glaucomatous visual field damage of approximately 1.2% was seen in patients with an IOP ranging between 6 mm Hg and 14 mm Hg. In 1987, Flammer et al. first suggested the beneficial effect of oral nifedipine, a calcium antagonist, on glaucomatous visual field damage in a subset of open angle glaucoma patients.40,41 Although not always confirmed,42 beneficial effects of oral calcium antagonists on visual field in some NTG patients have been reported by several investigators.6-9 The mechanism of action of calcium antagonists is not clear at present, but it may be related to their vasodilating effect in the central nervous system; according to Kitazawa et al. and Sawada et al, better recovery of skin temperature after cold exposure is a prognostic indicator of better retention of visual field in NTG patients treated with a calcium antagonist. Nilvadipine shows little effect on the systemic blood pressure in subjects without hypertension,43 which was also the case in the present subjects. This characteristic may be advantageous in the treatment of ophthalmologic diseases such as glaucoma.

References


