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Effect of Topically Applied Iganidipine Dihydrochloride, a Novel Calcium Antagonist, on Optic Nerve Head Circulation in Rabbits

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Purpose: To study the effect of topically applied iganidipine dihydrochloride (iganidipine), a novel water-soluble calcium channel blocker, on blood flow in the optic nerve head (ONH), intraocular pressure, and systemic blood pressure in rabbits.

Methods: After 0.1% iganidipine (20 μL) was instilled into normal eyes, the change in ONH blood flow was measured using a hydrogen gas clearance flowmeter. Iganidipine (0.0001% to 0.1%) was instilled into eyes with impaired ocular circulation before or after the intravitreal injection of endothelin-1, and the change in ONH blood flow was measured. Changes in intraocular pressure and blood pressure after instillation of 0.1% iganidipine were measured. In all experiments, physiological saline was instilled into the contralateral eye as a control.

Results: Iganidipine significantly increased the ONH blood flow in normal eyes with the maximum increment of 31.7% at 45 minutes after instillation. Preinstillation of 0.01% and 0.1% iganidipine significantly inhibited the decrease in ONH blood flow in the eyes with impaired circulation. Moreover, ONH blood flow recovered with postinstillation of 0.1% iganidipine. These effects were persistent. Instillation of 0.1% iganidipine did not change either the intraocular pressure or the blood pressure.

Conclusion: The instillation of iganidipine persistently increased and maintained the ONH blood flow in rabbit eyes with normal and impaired ocular circulation. **Jpn J Ophthalmol 2001;45:76–83**

Key Words: Blood flow in ONH, calcium channel blocker, endothelin-1, iganidipine dihydrochloride, instillation.

Introduction

Apart from intraocular pressure, impairment of blood flow in the optic nerve head (ONH) is believed to be one of the significant etiological factors in the development of normal-tension glaucoma (NTG).[1,2] Recently, the endogenous vasoconstrictor, endothelin-1 (ET-1) has attracted interest as a possible causal agent of blood flow impairment.[3] Levels of ET-1 in patients with NTG are higher than those in normal individuals.[4–6] The intravitreal injection of ET-1 in rabbits leads to constriction of the retinal vasculature,[7,8] reduction of blood flow in the ONH and choroid,[5,9] and prolongation of the latency of visual evoked potentials.[5] These findings have led to the inference that ET-1 plays a role in NTG and that impaired ocular circulation may be a causal factor for NTG. It has also been reported that oral calcium antagonists (Ca-antagonists) improve visual field impairment or prevent its progression in patients with NTG.[10,11] Other research involving rabbits and cats has shown that intravenous nicardipine increased blood flow in the retina and ONH, respectively.[12,13] Hence, Ca-antagonists and other circulation-improv-

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ing drugs may be useful in treating NTG. Because glaucoma is an ocular disease with chronic progression and medication must be taken over a long period, ongoing treatment with systemic agents will be problematic because of the development of secondary adverse reactions, such as hypotension. Additionally, with hypotension causing lowered ocular perfusion pressure, there is also the risk that blood flow in the ONH will further decrease, which would be undesirable.[14] On the other hand, because most dihydropyridine Ca-antagonists developed to date have been insoluble in water, their development as aqueous eyedrops for topical therapy has been difficult even though their administration as eyedrop preparations would be preferable.

Iganidipine dihydrochloride (iganidipine) is a dihydropyridine derivative Ca-antagonist synthesized by the Kyoto Pharmaceutical Company, with a more potent and sustained vasodilatory action than nicardipine.[15] In addition, unlike conventional dihydropyridine Ca-antagonists, iganidipine is extremely water soluble.[16] In the present study, therefore, we prepared an aqueous solution of iganidipine as eyedrops, and investigated the effects on ONH blood flow in normal rabbit eyes as well as on reduced ONH blood flow in eyes with impaired ocular circulation. Its effects on intraocular pressure and systemic blood pressure were also evaluated.

Materials and Methods

Male Dutch rabbits (Fukuzaki Rabbit-breeding Cooperative, Kanzaki, Hyogo) weighing 1.8–2.5 kg were housed in an animal room maintained at 24 ± 4°C, and 55 ± 15% humidity (reference value ± allowable range). Healthy animals without any ocular abnormalities were used in the experiments. Rabbits were fed pellets (Labo MR Stock, Nihon Nosan, Yokohama) at a rate of 100 g per day, with water was allowed ad libitum. Animals were handled in accordance with the ARVO Statement on the Use of Animals in Vision Research.

Iganidipine (bulk drug supplied by Kyoto Pharmaceutical, 3-(4-allyl-1-piperazinyl)-2,2-dimethylpropyl methyl-1,4-dihydro-2,6-dimethyl-4-(3-nitrophenyl)-3,5- pyridinecarboxylate dihydrochloride; MW 599.6) is a dihydropyridine derivative with the structural formula shown in Figure 1. For the purposes of this study, iganidipine was dissolved in a vehicle containing 0.1% sodium acetate (Nacalai Tesque, Kyoto), 0.05% benzalkonium chloride (Nacalai Tesque), and 0.9% sodium chloride (Nacalai Tesque) in sterile purified water; the pH was adjusted to 5 with acetic acid (Nacalai Tesque), making a final concentration of 0.1%. This solution was diluted as required to the desired concentration with the vehicle adjusted to a pH of 5. Endothelin-1 (Human, Peptide Institute, Osaka), the agent used to reduce ocular blood flow, was completely dissolved in 0.1% acetic acid and diluted to a concentration of $10^{-}4$ M, then the concentration was adjusted to $10^{-}6$ M with Opeguard MA® (Senju Pharmaceutical, Osaka).

Experiment 1-1: Effect of Iganidipine on ONH Blood Flow in Normal Eyes

Six rabbits were used. Urethane (1 g/kg) was injected subcutaneously in the abdominal region to induce general anesthesia. About an hour later, blood flow in the ONH was measured in animals under a stable level of anesthesia, using a hydrogen clearance flowmeter (DHM-3001; M.T. Giken Company Ltd, Tokyo), in accordance with the technique described by Sugiyama et al.[17] For determination of a stable level of anesthesia, blood pressure was monitored as described in Experiment 3. When it was not stable, an appropriate dose of urethane was additionally injected. The region of the incision and implanted electrode sites were carefully sealed with Aronalpha surgical adhesive (Aronalpha A®; Sankyo, Tokyo); then, after confirming that the Aronalpha was completely dry and sealed, 20 μL of 0.1% iganidipine solution was instilled into 1 eye, and 20 μL of saline solution, into the contralateral eye (control eye). Blood flow was measured before instillation of iganidipine solution, at 15-minute intervals until 90 minutes after instillation, and thereafter at 30-minute intervals until 180 minutes after instillation. Blood flow values were expressed relative to an initial value defined as 100%, and the effects due to the instillation of iganidipine solution were studied and compared with the control eye.

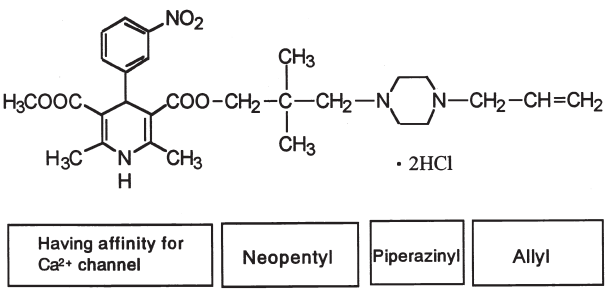

Figure 1. Structural formula of iganidipine.

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Experiment 1-2: Effect of Iganidipine Pretreatment in Eyes with Impaired Circulation

Twenty-five rabbits were used; 10 μL of ET-1 at a concentration of 10^{-6} M was injected into the center of the vitreous of each eye, using a microsyringe fitted with a 30-G needle, and monitoring progress of the needle's tip from the pupil region. One hour before injection of ET-1, 20 μL of iganidipine (0.1, 0.01, 0.001 or 0.0001%) was instilled into 1 eye. Saline solution was similarly instilled into the contralateral eye (control eye). The ONH blood flow was measured according to the method described in Experiment 1-1 before instillation of iganidipine, at 30 and 60 minutes (immediately before injection of ET-1) after instillation, and thereafter at 30-minute intervals until 180 minutes after injection of ET-1. In order to comprehensively evaluate the inhibitory effects of iganidipine on the reduction in blood flow, the area over the curve and under the line of 100% from the time of ET-1 injection until 180 minutes after injection (Area) was determined when the relative blood flow at ET-1 injection was 100%, and the inhibitory rate (%) was calculated using the formula: {1−(Area for iganidipine eye/Area for control eye)}× 100.

Experiment 1-3: Effect of Iganidipine Posttreatment in Eyes with Impaired Circulation

Six rabbits were used. The ET-1 was injected in the manner described in Experiment 1-2. At 30 minutes after injection of ET-1, ONH blood flow was confirmed to have decreased by the same amount in both eyes, then 20 μL of iganidipine solution was instilled into 1 eye and saline solution similarly instilled into the contralateral eye (control eye). The ONH blood flow was measured according to the technique described in Experiment 1-1 before and at 30 minutes (immediately before instillation of iganidipine) after injection of ET-1, and thereafter at 30-minute intervals until 210 minutes after instillation of iganidipine.

Experiment 2: Effect of Iganidipine on Intraocular Pressure

Eight rabbits were used. Intraocular pressure was measured using an applanation pneumatonometer (Alcon Labs, Fort Worth, TX, USA), with local anesthesia provided by instillation of 0.04% oxybuprocaine hydrochloride (Anelocal®; Senju). Twenty microliters of 0.1% iganidipine solution were instilled into 1 eye and 20 μL of saline solution into the contralateral eye. Intraocular pressure was measured before instillation, and at 30 minutes, and 1, 2, and 4 hours after instillation of iganidipine solution.

Experiment 3: Effect of Iganidipine on Blood Pressure

Six rabbits were used. Blood pressure was measured in rabbits under urethane anesthesia, via a blood pressure gauge (AP-641G; Nihon Kohden, Tokyo) connected to a pressure transducer (TP-200T, Nihon Kohden) in a 24-G indwelling needle (Terumo, Tokyo) implanted into an auricular artery. Twenty microliters of 0.1% iganidipine solution were instilled into 1 eye and 20 μL of saline solution into the contralateral eye. Blood pressure was measured before instillation of iganidipine, at 15-minute intervals up to 1 hour after instillation, and thereafter at 1-hour intervals until 3 hours after instillation.

Statistical Analysis

Results are presented as mean ± SE. Statistical analysis was performed by two-way analysis of variance (ANOVA) for repeated measurements of ONH blood flow. If a statistically significant difference was detected, further assessment was performed by the paired *t*-test with correction for multiplicity provided by Bonferroni's method. Differences from baseline values for intraocular pressure and blood pressure were tested for significance by the paired *t*-test with correction by Bonferroni's method. Differences were regarded as significant when $P < .05$.

Results

Experiment 1-1: Effect of Iganidipine on ONH Blood Flow in Normal Eyes

The baseline optic disc blood flow measurements (mL/min per 100 g, mean ± SE, n = 6) in iganidipine and control eyes were 38.3 ± 7.3 and 38.6 ± 2.9, respectively. Statistical analysis showed no significant difference between the two.

The effect of iganidipine on ONH blood flow is shown in Figure 2. Two-way ANOVA for repeated measurements revealed a significant difference between iganidipine and control eyes. At 15, 30, 60 and 75 minutes after instillation of 0.1% iganidipine solution, ONH blood flow in the iganidipine eyes was significantly higher than in the control eyes. The maximum increment of 31.7% was recorded at 45 minutes after instillation.

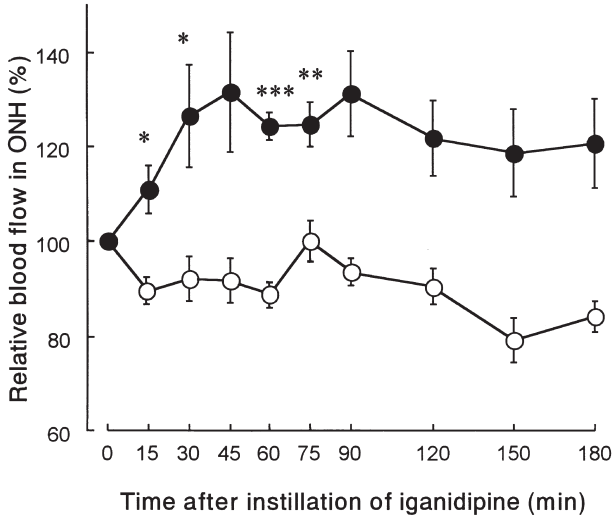

Figure 2. Changes in optic nerve head (ONH) blood flow after instillation of 0.1% iganidipine in normal eyes. ●: Iganidipine-treated eyes, ○: fellow control eyes. Each plot represents mean (± SE) of results obtained in 6 rabbits. There was significant difference between iganidipine and control eyes ($P = .0006$; two-way analysis of variance for repeated measurements). Asterisks indicate significant differences from controls (*$P < .05$, **$P < .01$, ***$P < .001$; paired *t*-test corrected by Bonferroni's method).

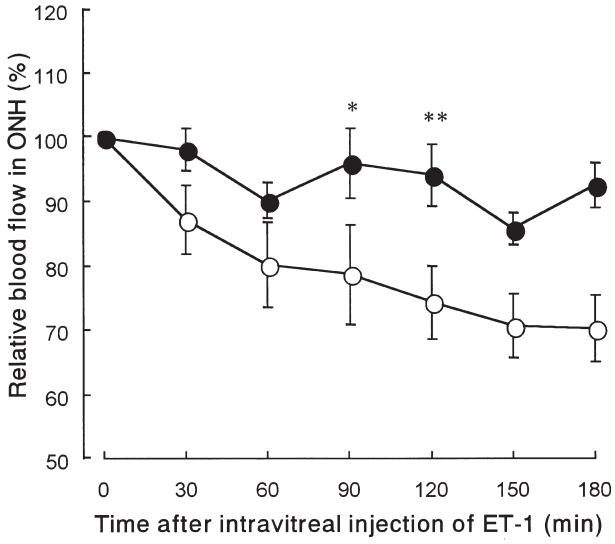

Figure 3. Changes in optic nerve head (ONH) blood flow after preinstillation of 0.1% iganidipine in eyes with impaired ocular circulation. Each plot represents mean (± SE) of results obtained in 7 rabbits. For explanation of symbols, see legend for Figure 2. There was significant difference between iganidipine and control eyes ($P = .0284$; two-way analysis of variance for repeated measurements).

Experiment 1-2: Effect of Iganidipine Pretreatment in Eyes with Impaired Circulation

The baseline blood flow measurements (mL/min per 100 g, mean ± SE) in iganidipine and control eyes were 46.1 ± 3.8 and 46.6 ± 4.0, respectively, in the 0.1% group (n = 7); 58.2 ± 5.7 and 60.2 ± 7.0 in the 0.01% group (n = 6); 60.7 ± 8.2 and 82.7 ± 14.3 in the 0.001% group (n = 6); and 53.9 ± 4.5 and 48.8 ± 4.6 in the 0.0001% group (n = 6). There was no significant difference between iganidipine and control eyes in any group.

In the control eyes, ONH blood flow decreased from the baseline value by a mean of about 20% at 30 minutes after injection of ET-1 in each experiment, and thereafter declined gradually. At 180 minutes after injection of ET-1, blood flow decreased by a maximum of about 34%. In the eyes instilled with 0.1% iganidipine, ONH blood flow increased by about 10% before ET-1 injection. The increment was apparently lower than in Experiment 1-1, but the difference was not significant. The effect of 0.1% solution on the decrease in ONH blood flow is shown in Figure 3. Two-way ANOVA for repeated measurements revealed a significant difference between iganidipine and control eyes. At 90 and 120 minutes after injection of ET-1, the 0.1% iganidipine solution inhibited the decrease in ONH blood flow significantly, maintaining blood flow at levels not significantly lower than the baseline value.

The effect of 0.01% iganidipine solution on the decrease in ONH blood flow is shown in Figure 4.

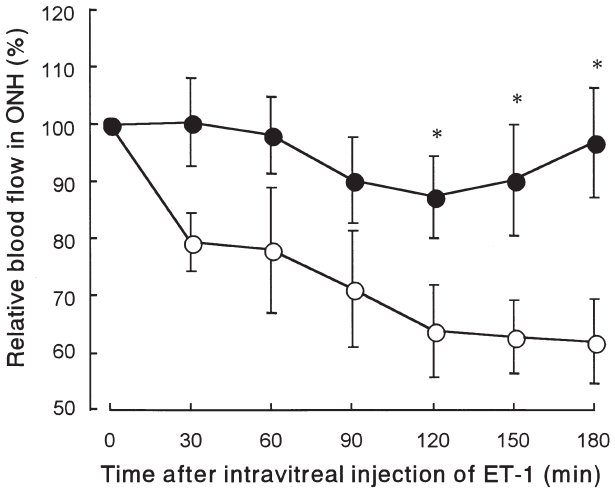

Figure 4. Changes in optic nerve head (ONH) blood flow after preinstillation of 0.01% iganidipine in eyes with impaired ocular circulation. For explanation of symbols, see legend for Figure 2. There was significant difference between iganidipine and control eyes ($P = .0347$; two-way analysis of variance for repeated measurements).

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Two-way ANOVA for repeated measurements revealed a significant difference between iganidipine and control eyes. Iganidipine inhibited the ET-1-induced decrease in ONH blood flow significantly at 120, 150, and 180 minutes after injection of ET-1, with higher levels of ONH blood flow measured, and maintained blood flow at levels not significantly lower than the baseline value.

The ONH blood flow in the treated eyes with 0.001% solution was greater than in the control eyes (data not shown), but the inhibitory effect on the decrease was not significant (P = .156; two-way ANOVA for repeated measurements). The changes in ONH blood flow in eyes instilled with a 0.0001% iganidipine solution after injection of ET-1 were similar to those in the control eyes (P = .9433; two-way ANOVA for repeated measurements).

The inhibitory rate for each concentration in the iganidipine-pretreated eyes, relative to the decrease in ONH blood flow in the control eyes, is shown in Figure 5. It is almost dose-dependent although the values for 0.01% and 0.1% solutions are not significantly different (P=.3233, unpaired t-test).

Experiment 1-3: Effect of Iganidipine Posttreatment in Eyes with Impaired Circulation

The baseline ONH blood flow values (mL/min per 100 g, mean ± SE, n = 6) in the iganidipine posttreatment eyes and control eyes were not significantly different, at 42.7 ± 2.3 and 46.7 ± 5.7, respectively. At 30 minutes after injection of ET-1, ONH blood flow decreased in both eyes by about 29% from the pre-injection values. From the time of ET-1 instillation onward, the maximum decrease in the control eye was 37.0% at 60 minutes after instillation, and the decrease persisted until 210 minutes after instillation. In the eyes treated with 0.1% iganidipine solution, the ET-1-induced lower blood flow had practically returned to its baseline level by 30 minutes after iganidipine instillation. Two-way ANOVA for repeated measurements revealed a significant difference between iganidipine and control eyes. At 60, 90, 150, 180, and 210 minutes after instillation of iganidipine, the 0.1% solution significantly inhibited the decrease in ONH blood flow (Figure 6).

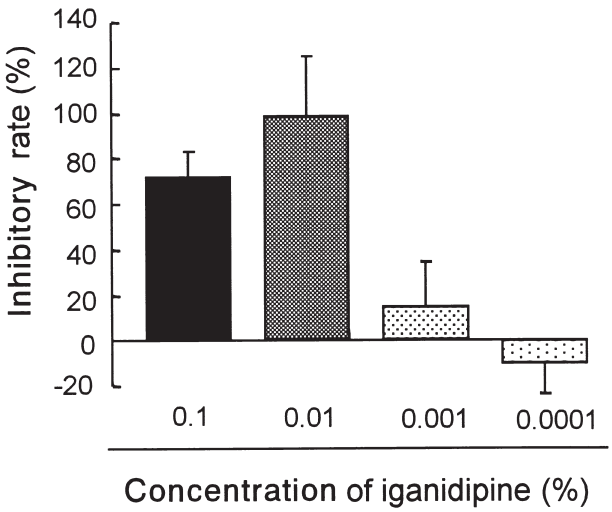

Figure 5. Inhibitory effects of preinstillation of iganidipine at various concentrations on decrease in optic nerve head blood flow in eyes with impaired circulation. Each value represents mean (± SE) of results obtained in 6–7 rabbits.

Experiment 2: Effect of Iganidipine on Intraocular Pressure

The intraocular pressure values (mm Hg, mean ± SE, n = 8) in the iganidipine eyes and control eyes before instillation and at 30 minutes, 1, 2, and 4 hours after instillation of 0.1% iganidipine solution were (27.3 ± 1.0, 26.6 ± 0.4), (27.4 ± 1.0, 25.4 ± 0.5), (27.4 ± 0.8, 26.8 ± 0.5), (27.8 ± 0.6, 26.9 ± 0.6) and (26.4 ± 1.0, 26.1 ± 0.7), respectively. Thus, the intraocular pressure values in both eyes at all measurement times were practically unchanged.

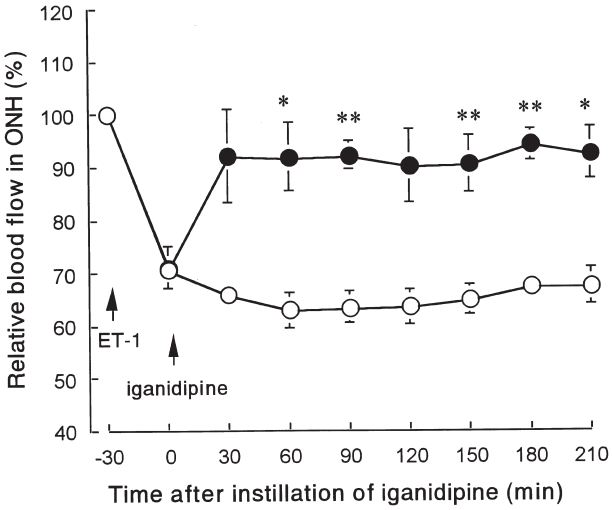

Figure 6. Changes in optic nerve head (ONH) blood flow after postinstillation of 0.1% iganidipine in eyes with impaired ocular circulation. For explanation of symbols, see legend for Figure 2. There was significant difference between iganidipine and control eyes (P = .0001; two-way analysis of variance for repeated measurements).

Experiment 3: Effect of Iganidipine on Blood Pressure

While the baseline blood pressure was 92.9 ± 4.6 mm Hg (mean ± SE, n = 6), the blood pressure readings at 15, 30, and 45 minutes, and at 1, 2, and 3 hours after instillation of 0.1% iganidipine solution were 96.7 ± 6.1, 91.4 ± 4.7, 90.9 ± 4.4, 90.9 ± 4.8, 93.4 ± 4.0, and 96.5 ± 6.2, respectively. Thus, the mean blood pressure values at all measurement times were not significantly changed.

Discussion

We investigated the effects of an eye-drop formulation of iganidipine, a new Ca-antagonist, on ONH blood flow in normal rabbit eyes and on decreased optic disc blood flow in an experimental model of impaired ocular circulation. Rabbits were used as the test animals in this study, for ease of handling, and because the vascular architecture of the ONH in this species has been clearly elucidated.[18] Blood flow was measured by the established hydrogen clearance technique.[19–22]

The ONH blood flow decreased slightly in the normal control eyes instilled with saline solution, possibly due to the effect of general anesthesia, injury by the electrode or other factors. Instillation of 0.1% iganidipine solution caused a significant increase in ONH blood flow by the maximum increment of about 30%, although it did not induce any changes in intraocular pressure or blood pressure. Other studies have shown that iganidipine has a potent vasorelaxant effect, owing to its Ca-antagonistic action, on isolated canine cerebral artery, coronary artery, mesenteric artery, and renal artery,[15] and that in normotensive rats, iganidipine acts to increase blood flow in the brain, brown adipose tissue, intestine, colon, and skin.[23] Hence, like its effects in other tissues, iganidipine may increase blood flow in the ONH vasculature by exerting a potent vasorelaxant effect in spite of the presence of an automatic regulatory mechanism for adjusting the ONH blood flow.[24,25]

Another cause of increased ONH blood flow in normal rabbit eyes may be due to the chemical characteristics of the compound. In brief, iganidipine is extremely water soluble because it has a hydrophilic piperazinyl group and is 91% dissociated at physiological pH levels, while the presence of lipophilic neopentyl and allyl groups means that it is also highly lipid soluble.[16] Therefore, iganidipine seems to have excellent tissue permeability, which would probably result in rapid intraocular distribution of instilled iganidipine; its distributed concentration is now under investigation. Other studies[26,27] regarding betaxolol, which also has calcium channel blocking properties, have shown that betaxolol penetrates the conjunctiva and accumulates in the Tenon capsule and that betaxolol reaches the retina in maximal amounts within 60 minutes. These studies suggest that iganidipine might also penetrate through the periocular tissue to reach the vessels in ONH, then affect them rapidly after instillation.

The ET-1 activates ET-1 receptors on the cellular membranes of vascular smooth muscle. This stimulates the entry of Ca^{2+} ions through calcium channels and the release of Ca^{2+} ions from sarcoplasmic reticulum, and the increase in intracellular Ca^{2+} concentration induces muscular contraction by activating myosin light chain kinase.[28] This contraction of vascular smooth muscle reduces the vascular bore and thereby decreases blood flow. Reduction in ocular blood flow has been suggested as one of the factors etiologically linked with glaucoma,[2,6,29,30] and in particular, ET-1 may be one of the responsible factors for decreasing blood flow in NTG.[4–6] Our investigation of the effects of the instillation of iganidipine on the persistent decrease in ONH blood flow induced by intravitreal injection of ET-1 showed that iganidipine eyedrops dose-dependently inhibited the ET-1-induced decrease in ONH blood flow, and that the inhibition was practically complete at concentrations of 0.01% or more. The inhibitory effects of 0.1% and 0.01% were almost the same, suggesting that the maximum effect of iganidipine against ET-1 would be produced by instillation of the 0.01% solution. Earlier studies have shown that iganidipine induces relaxation (IC_{50}: 5×10^{-10} M) in specimens of isolated canine mesenteric artery in which contraction had been elicited by ET-1,[15] and that in normotensive rats, iganidipine inhibits ET-1-induced decreases in blood flow in lung, kidney, brown adipose tissue, and muscle,[23] and that these outcomes are due to the blocking of voltage-dependent Ca^{2+}-channels on cellular membranes in vascular smooth muscle, thereby inhibiting the ET-1-induced entry of extracellular Ca^{2+} into cells via Ca^{2+}-channels.[15] Hence, our finding that treatment with instilled iganidipine solution inhibited the ET-1-induced decrease in ONH blood flow could be because iganidipine was distributed to the ONH region, and was blocking the Ca^{2+}-channels in the vascular smooth muscle of the ONH that had been activated by ET-1.

When iganidipine was instilled after blood flow had decreased by about 30% in our experimental model of impaired ocular circulation, blood flow re-

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covered rapidly and the effect was sustained for an extended period. From this result it could be concluded that instillation of iganidipine may even increase blood flow in a pathological state of reduced circulation. This result leads to the suggestion that iganidipine may be therapeutically effective in eye diseases in which diminished ocular blood flow is a characteristic.

The effects of iganidipine on ONH blood flow in normal and ET-1-treated eyes were persistent in the present study. In research involving spontaneously hypertensive rats, iganidipine administered by a single intravenous injection caused a persistent reduction in blood pressure that lasted for 4–7 hours.[31] In isolated canine mesenteric arteries, the tissue concentration of iganidipine rose to levels more than 20 times higher than that in the medium, and even after repeated washouts, elimination of the drug from the tissue was slow, demonstrating that iganidipine has a protracted duration of action on blood vessels.[15] This outcome was thought to be due to the slow dissociation of the binding between iganidipine and Ca^{2+}-channels.[16] From these findings, we can hypothesize that the sustained action of iganidipine in the present study may be caused by the slow dissociation of the specific binding between iganidipine and Ca^{2+}-channels also in vascular smooth muscle in the ONH.

On the basis of the results presented above, it can be concluded that iganidipine topically applied to the eye produces a potent and sustained action of increasing blood flow in the ONH by dilating blood vessels via a mechanism involving its binding to L-type Ca^{2+}-channels in vascular smooth muscle in the ONH. This leads to the expectation that iganidipine might be useful in treating the ocular diseases, including NTG, in which impaired circulation is one of the causal factors.

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