inhibit contraction of the residual lens capsule has been proposed.[8,18] Long-term preservation of the capsular bag diameter or shape has been demonstrated in cases with the in-the-bag IOL, but not in cases without IOL implantation or with the IOL out-of-the-bag implantation.[19]

Another possible potential theory about the pathogenesis of retinal detachments in aphakic eyes is that the forward displacement of the vitreous with blinking and rapid eye movements may contribute to the development of retinal detachment. However, the causative retinal breaks located at the pars plana or the ora seratta with features of retinal detachment associated with atopic dermatitis were different from the retinal breaks detected in the aphakic eyes.

Yttrium–aluminum–garnet posterior capsulotomy did not affect the incidence of retinal detachment, but a PCR during cataract surgery increased the risk for retinal detachment associated with giant breaks. Anterior vitrectomy may induce an anterior loop contraction of the vitreous base, resulting in development of giant breaks at the ora serrata.

We conclude that cataract extraction and IOL implantation with in-the-bag fixation, which keeps the posterior capsule intact, seems to have the advantage of decreasing the incidence of postoperative retinal detachment. Additional studies to determine whether an encircling buckling or complete vitrectomy will prevent retinal detachments in patients with PCR will be necessary.

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Suppression of Ocular Inflammation in Endotoxin-Induced Uveitis by Blocking the Angiotensin II Type 1 Receptor

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Purpose. To examine whether the angiotensin II type 1 receptor (AT1-R) signaling plays a role in ocular inflammation in endotoxin-induced uveitis (EIU).

Methods. EIU was induced in C57BL/6 mice by a single intraperitoneal injection of 150 μg lipopolysaccharide (LPS). Tissue localization, mRNA expression, and protein levels of AT1-R in murine retinas were examined by immunohistochemistry, RT-PCR, and Western blot analyses, respectively. Telmisartan, an AT1-R antagonist widely used as an antihypertensive agent, was administered intraperitoneally at a dose of 10 mg/kg daily for 5 days until the injection of LPS. Twenty-four hours after administration, leukocyte adhesion to the retinal vasculature was evaluated with a concanavalin A lectin perfusion-labeling technique. Retinal mRNA and protein levels of intercellular adhesion molecule (ICAM)-1 were examined by RT-PCR and ELISA, respectively. Protein concentration and inflammatory cells in the aqueous humor were also measured.

Results. Retinal vessels were positive for AT1-R. In mice with EIU, retinal AT1-R mRNA and protein levels were significantly increased when compared to the normal control. EIU animals also showed significant increases in the number of inflammatory cells infiltrating the anterior chamber and adhering to the retinal vessels and in retinal ICAM-1 levels. Administration of telmisartan to EIU mice resulted in significant suppression of retinal ICAM-1 expression and leukocyte adhesion and infiltration compared with vehicle treatment. Protein concentration in the aqueous humor of telmisartan-treated EIU mice tended to be lower than that of vehicle-treated EIU mice, but the difference was not statistically significant.

Conclusions. AT1-R signaling blockade inhibited retinal ICAM-1 upregulation and leukocyte adhesion and infiltration in the EIU model. These results suggest the potential use of an AT1-R antagonist as a therapeutic agent to reduce ocular inflammation. (*Invest Ophthalmol Vis Sci.* 2005;46:2925-2931) DOI:10.1167/iovs.04-1476

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Endotoxin-induced uveitis (EIU) is an animal model of acute ocular inflammation induced by the administration of lipopolysaccharide (LPS), a component of Gram-negative bacterial outer membranes.[1-3] Because uveitis frequently leads to severe vision loss and blindness with retinal vasculitis, retinal detachment, and glaucoma, it is important to elucidate further the mechanisms in the development of ocular inflammation. LPS enhances the expression of various inflammatory mediators, such as interleukin (IL)-6,[3,4] tumor necrosis factor (TNF)-α,[5] prostaglandin E2,[6] and monocyte chemotactic protein (MCP)-1,[7] as well as the production of nitric oxide,[6] all of which contribute to the development of EIU, resulting in the breakdown of the blood-ocular barrier and in the infiltration of leukocytes. For the first phase of leukocyte infiltration, cell adhesion to vascular endothelium is essential, in which adhesion molecules play major roles.[8] Among various adhesion molecules, intercellular adhesion molecule (ICAM)-1 and its receptor, lymphocyte function-associated antigen (LFA)-1, are necessary for the development of EIU.[8-10] Although EIU was originally used as a model of anterior uveitis, increasing evidence shows that it also involves inflammation in the posterior segment of the eye with recruitment of leukocytes that adhere to the retinal vasculature and infiltrate the vitreous cavity.[11,12]

The renin-angiotensin system is a major controller of systemic blood pressure. Angiotensin II, the effector molecule of the system, has two cognate receptors: angiotensin II type 1 receptor (AT1-R) and AT2-R.[13,14] Because major functions of angiotensin II are mediated by AT1-R, its antagonists are widely used to treat patients with hypertension and cardiovascular diseases. Recently, several studies have demonstrated the diverse biological functions of angiotensin II as a modulator of angiogenesis, vascular remodeling, and inflammation.[15-20] As an inflammatory mediator, angiotensin II enhances vascular permeability through prostaglandins and vascular endothelial growth factor,[17] and contributes to the recruitment of inflammatory cells by inducing chemokines and adhesion molecules.[18,19] Moreover, angiotensin II directly induces the proliferation and differentiation of inflammatory cells per se.[20] AT1-R blockade is reported to attenuate such inflammatory processes effectively.[17-19] Recent studies have demonstrated the prevention of EIU by suppressing inflammatory mediators including IL-6, TNF-α, cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and MCP-1.[5-7,21-24] However, it is not clear whether AT1-R blockade is effective in reducing ocular inflammation. In the current study we show for the first time the anti-inflammatory effects of an AT1-R antagonist, telmisartan, on ocular inflammation in a murine model of EIU.

Methods

Animals and Induction of EIU

C57BL/6 mice (7-10 weeks old; SLC, Shizuoka, Japan) were used. All animal experiments were conducted in accordance with the ARVO

Statement for the Use of Animals in Ophthalmic and Vision Research. The ethics committee of our institution approved all surgical interventions and animal care procedures, which were in accordance with the Guidelines and Policies for Animal Surgery provided by the Animal Study Committees of the Central Institute for Experimental Animals of Keio University. Animals received a single intraperitoneal injection of 0.15 mg LPS from *Escherichia coli* (Sigma-Aldrich, St. Louis, MO) in 0.15 mL phosphate-buffered saline (PBS).

Pretreatment with Telmisartan

Telmisartan was a gift of Boehringer Ingelheim, Ingelheim, Germany. Animals were pretreated with 0.15-mL intraperitoneal injections of vehicle (0.25% dimethylsulfoxide [DMSO] in PBS) or telmisartan daily for 5 days until the injection of LPS. LPS was injected immediately after the fifth telmisartan injection. We dissolved the telmisartan in in 30 mM DMSO, diluted to 60 μM with PBS and injected into mice at a dose of 10 mg/kg body weight. This dose was sufficient to block AT1-R signaling to decrease systemic blood pressure in rats.[25] The effects of telmisartan pretreatment on ocular inflammation were evaluated 24 hours after LPS injection.

Lectin Labeling of Retinal Vasculature and Adherent Leukocytes

The retina-adherent leukocytes were imaged by perfusion labeling with fluorescein-isothiocyanate (FITC)-coupled concanavalin A lectin (con A; Vector, Burlingame, CA), as described previously.[26] In mice under deep anesthesia, the chest cavity was opened and a 27-gauge cannula was introduced into the left ventricle. After injection of 2 mL of PBS to remove erythrocytes and nonadherent leukocytes, 2 mL FITC-conjugated con A lectin was perfused. After the eyes were enucleated, the retinas were flatmounted. The flatmounts were imaged with an epifluorescence microscope (IX71; Olympus, Tokyo, Japan), and the total number of con A-stained adherent leukocytes per retina was determined.

Immunohistochemistry for AT1-R

Immunohistochemical experiments were performed with the murine eyes. For histopathologic evaluation, the specimen was fixed with 4% paraformaldehyde (PFA) at 4°C immediately after removal and embedded in paraffin. Three-micrometer paraffin sections were incubated overnight at 4°C with a rabbit polyclonal antibody against human AT1-R (Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:100 dilution. After incubation, they were reacted for 30 minutes at room temperature with goat antibodies against rabbit immunoglobulins (IgGs) conjugated to a peroxidase-labeled dextran polymer (En Vision+ rabbit; Dako Corp., Carpinteria, CA). As a negative control for staining, the first antibodies were replaced with nonimmune rabbit IgGs (Dako). Color was developed with DAB (3,3'-diaminobenzidine tetrahydrochloride; 0.2 mg/mL; Dojindo Laboratories, Kumamoto, Japan) in 0.05 M Tris-HCl (pH 7.6) containing 0.003% hydrogen peroxide, and the sections were counterstained with hematoxylin.

Aqueous Humor Analyses

Aqueous humor was collected by anterior chamber puncture with a 30-gauge needle at 0, 6, 12, 24, and 48 hours after LPS injection in vehicle- and telmisartan-treated EIU mice. Protein concentration was determined with a protein quantification kit (Dojindo Laboratories), and absorbance was measured with a microplate reader (Bio-Rad Laboratories, Hercules, CA). For evaluation of inflammatory cells in the anterior chamber, 1 μL of aqueous-humor samples were dropped on a poly-L-lysine-coated slide (Sigma-Aldrich) and air dried. Slides were processed with Wright's stain, and the total number of cells in each drop was counted under a light microscope, as described previously.[27]

RT-PCR Analyses

Total RNA was isolated from the retina and the iris-ciliary body complex with extraction reagent (TRIzol; Invitrogen, Carlsbad, CA) and reverse-transcribed with a cDNA synthesis kit (First-Strand; Amersham Biosciences, Inc., Piscataway, NJ) according to the manufacturer's protocols. PCR was performed with *Taq* DNA polymerase (Toyobo, Tokyo, Japan) in a thermal controller (MiniCycler; MJ Research, Watertown, MA). The primer sequences were as follows: 5'-ATG TGG CAC CAC ACC TTC TAC AAT GAG CTG CG-3' (sense) and 5'-CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC-3' (antisense; 837 bp) for β-actin; 5'-TCA CCT GCA TCA TCA TCT GG-3' (sense) and 5'-AGC TGG TAA GAA TGA TTA GG-3' (antisense; 204 bp) for mouse AT1-R; 5'-GTG TCG AGC TTT GGG ATG GTA-3' (sense) and 5'-CTG GGC TTG GAG ACT CAG TG-3' (antisense; 505 bp) for ICAM-1; 5'-TTC CTC TCT GCA AGA GAC T-3' (sense) and 5'-TGT ATC TCT CTG AAG GAC T-3' (antisense; 430 bp) for IL-6; 5'-AGC CCA CGT CGT AGC AAA CCA CCA A-3' (sense) and 5'-ACA CCC ATT CCC TTC ACA GAG CAA T-3' (antisense; 446 bp) for TNF-α; 5'-TGC ATG TGG CTG TGG ATG TCA TCA A-3' (sense) and 5'-CAC TAA GAC AGA CCC GTC ATC TCC A-3' (antisense; 449 bp) for COX-2; 5'-TCA CGC TTG GGT CTT GTT CAC T-3' (sense) and 5'-TTG TCT CTG GGT CCT CTG GTC A-3' (antisense; 472 bp) for iNOS; and 5'-ATC CCA ATG AGT AGG CTG GAG AG-3' (sense) and 5'-CAG AAG TGC TTG AGG TGG TTG TG-3' (antisense; 617 bp) for MCP-1.

Western Blot Analysis for AT1-R

The animals were killed with an overdose of anesthesia, and the eyes were immediately enucleated. The retina and the iris-ciliary body complex were carefully isolated and placed into 200 μL of lysis buffer (0.02 M HEPES, 10% glycerol, 10 mM $Na_4P_2O_7$, 100 μM Na_3VO_4, 1% Triton, 100 mM NaF, and 4 mM EDTA [pH 8.0]) supplemented with protease inhibitors (2 mg/L aprotinin, 100 μM phenylmethylsulfonyl fluoride, 10 μM leupeptin, and 2.5 μM pepstatin A) and sonicated. The lysate was centrifuged at 15,000 rpm for 15 minutes at 4°C, and the supernatants were collected and mixed with sample buffer. Each sample containing 50 μg of total protein was then boiled for 5 minutes, separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE, and electroblotted to polyvinylidene fluoride (PVDF) membrane (Millipore Corp., Bedford, MA). After nonspecific binding was blocked with 5% bovine serum albumin, the membranes were incubated with a rabbit anti-human AT1-R polyclonal antibody (1:100; Santa Cruz Biotechnology) at room temperature for 1 hour, followed by incubation with a horseradish-peroxidase-conjugated goat antibody directed against rabbit IgGs (1:5000; BioSource, Camarillo, CA). The signals were visualized with an enhanced chemiluminescence kit (ECL; Amersham Biosciences, Inc.) according to the manufacturer's protocol.

Enzyme-Linked Immunosorbent Assay for ICAM-1

The animals were killed with an overdose of anesthesia, and the eyes were immediately enucleated. The retina was carefully isolated and placed into 200 μL of lysis buffer supplemented with protease inhibitors and sonicated. The lysate was centrifuged at 15,000 rpm for 15 minutes at 4°C, and the ICAM-1 level in the supernatant was determined with the mouse ICAM-1 kit (R&D Systems Inc., Minneapolis, MN) according to the manufacturer's protocol. The tissue sample concentration was calculated from a standard curve and corrected for protein concentration.

Morphometric and Statistical Analyses

All results are expressed as the mean ± SD. The number of leukocytes in each flatmount was counted independently by two investigators with the epifluorescence microscope. The data were processed for statistical analyses (Mann-Whitney test). Differences were considered to be statistically significant at $P < 0.05$.

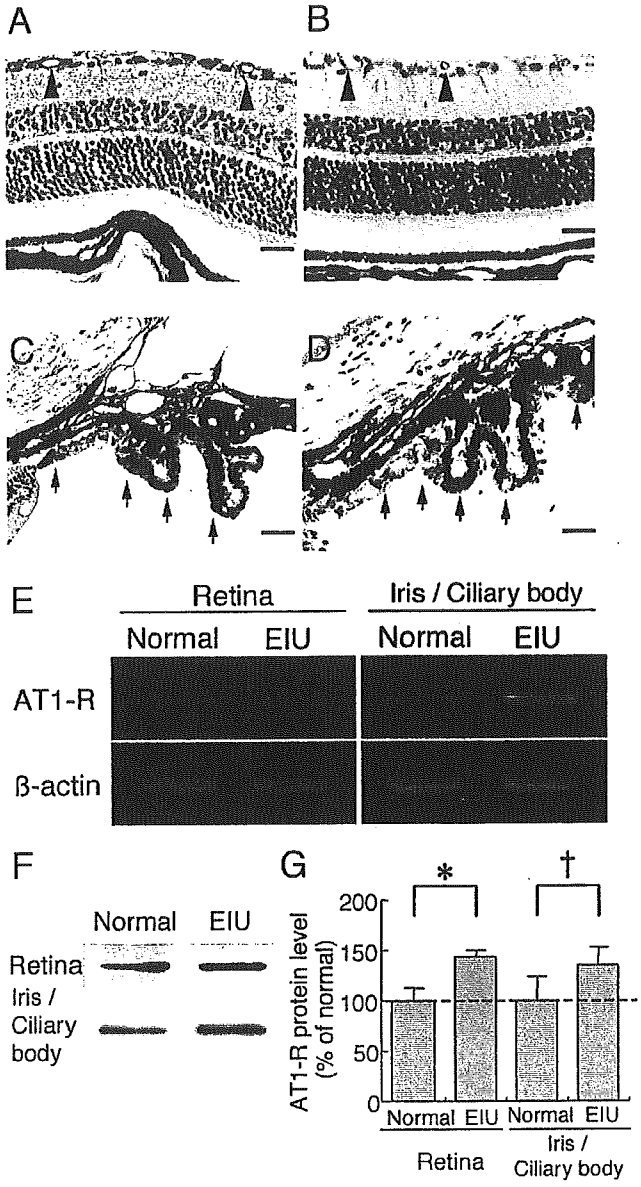

FIGURE 1. AT1-R tissue localization and induction in EIU. Immunohistochemical staining (A–D), RT-PCR (E), and Western blot analysis (F, G) of AT1-R expression in the retina and the iris–ciliary body complex. Retinal sections from normal (A) and EIU (B) mice. Positive staining for AT1-R on the inner retinal vessels (A, B, *arrows*). Sections of iris–ciliary body complex from normal (C) and EIU (D) mice. Positive staining for AT1-R on the nonpigmented epithelial cells of the ciliary body (C, D, *arrows*). Scale bar, 100 μm. AT1-R mRNA (E) and protein (F, G) levels of the retina and iris-ciliary body in EIU mice were higher than those in normal age-matched mice. The results represent the mean ± SD; $n = 6$. $^*P < 0.01$, $^\dagger P < 0.05$, by Mann-Whitney test.

RESULTS

AT1-R Tissue Localization and Upregulation in EIU

Immunohistochemistry for AT1-R was performed to identify its expression in the eyes in normal (Figs. 1A, 1C) and EIU (Figs. 1B, 1D) mice. AT1-R immunoreactivity was detected mainly on the endothelial cells of inner retinal vessels (Figs. 1A, 1B) and nonpigmented epithelial cells of the ciliary body (Figs. 1C, 1D). Retinas and iris–ciliary body complex were subjected to RT-PCR and Western blot analyses to detect the expression of AT1-R at mRNA (Fig. 1E) and protein (Figs. 1F, 1G) levels, respectively. AT1-R mRNA and protein levels of the retina and the iris–ciliary body complex in EIU mice were significantly higher than in normal, age-matched animals.

Effects of Telmisartan on Retinal Leukocyte Adhesion

The retina-adherent leukocytes were imaged by perfusion labeling with FITC-coupled con A. Leukocyte counts were evaluated in the posterior retina around the optic disc (Figs. 2A–D), the midperipheral retina near the equator of the globe (Figs. 2E–H), and the peripheral (anterior) retina next to the ora serrata (Figs. 2I–L). Retina-adherent leukocytes, which were few in normal mice (Figs. 2A, 2E, 2I), increased with induction of EIU (Figs. 2B, 2F, 2J). Compared with vehicle-treated EIU retina (Figs. 2C, 2G, 2K), telmisartan administration (Figs. 2D, 2H, 2L) suppressed leukocyte adhesion in the EIU retina. The total number of adherent leukocytes in nontreated EIU mice (125.3 ± 30.5) was significantly ($P < 0.01$) higher than in normal age-matched control animals (4.4 ± 2.1). Telmisartan-treated EIU mice showed a significant ($P < 0.01$) decrease in leukocyte counts (to 62.5 ± 12.0), compared with vehicle-treated EIU mice (to 116.0 ± 20.1) or nontreated EIU mice (to 125.3 ± 30.5) (Fig. 2M).

Effects of Telmisartan on Retinal ICAM-1 Expression

Retinal ICAM-1 expression at mRNA and protein levels was analyzed by RT-PCR (Fig. 3A) and ELISA (Fig. 3B), respectively. Retinal ICAM-1 mRNA expression in nontreated and vehicle-treated EIU mice was higher than in normal age-matched control animals. Systemic administration of telmisartan substantially reduced ICAM-1 mRNA expression (Fig. 3A). Similarly, retinal ICAM-1 protein levels were significantly attenuated after pretreatment with telmisartan ($P < 0.05$, Fig. 3B).

Effects of Telmisartan on Retinal Expression of Inflammatory Mediators

To determine whether telmisartan affects inflammatory mediators associated with the pathogenesis of EIU, we analyzed retinal mRNA expression of IL-6, TNF-α, COX-2, iNOS, and MCP-1 at 6 hours after LPS injection by RT-PCR (Fig. 4). Retinal mRNA expressions of these agents in vehicle-treated EIU mice were higher than in normal age-matched control mice. Systemic administration of telmisartan substantially reduced expression of their mRNA.

Effects of Telmisartan on Anterior Chamber Protein Leakage and Leukocyte Infiltration

Telmisartan pretreatment led to suppression of leukocyte adhesion to the retinal vasculature in the posterior to anterior (peripheral) region, showing its anti-inflammatory effects on the posterior and intermediate segments of the globe. To evaluate its anti-inflammatory effect on anterior uveitis, we analyzed protein leakage and leukocyte infiltration into the aqueous humor. Protein concentration and leukocyte counts in the aqueous humor of the telmisartan-treated EIU mice were compared with that in vehicle-treated EIU mice (Fig. 5). Telmisartan-treated EIU mice showed a significant ($P < 0.01$) decrease in the cell counts at 12 and 24 hours after LPS injection, compared with vehicle-treated EIU mice (Fig. 5A). Protein concentration in the aqueous humor of the telmisartan-treated EIU mice at 12 and 24 hours after LPS injection tended to be lower than that of vehicle-treated EIU mice, but the difference was not statistically significant (Fig. 5B).

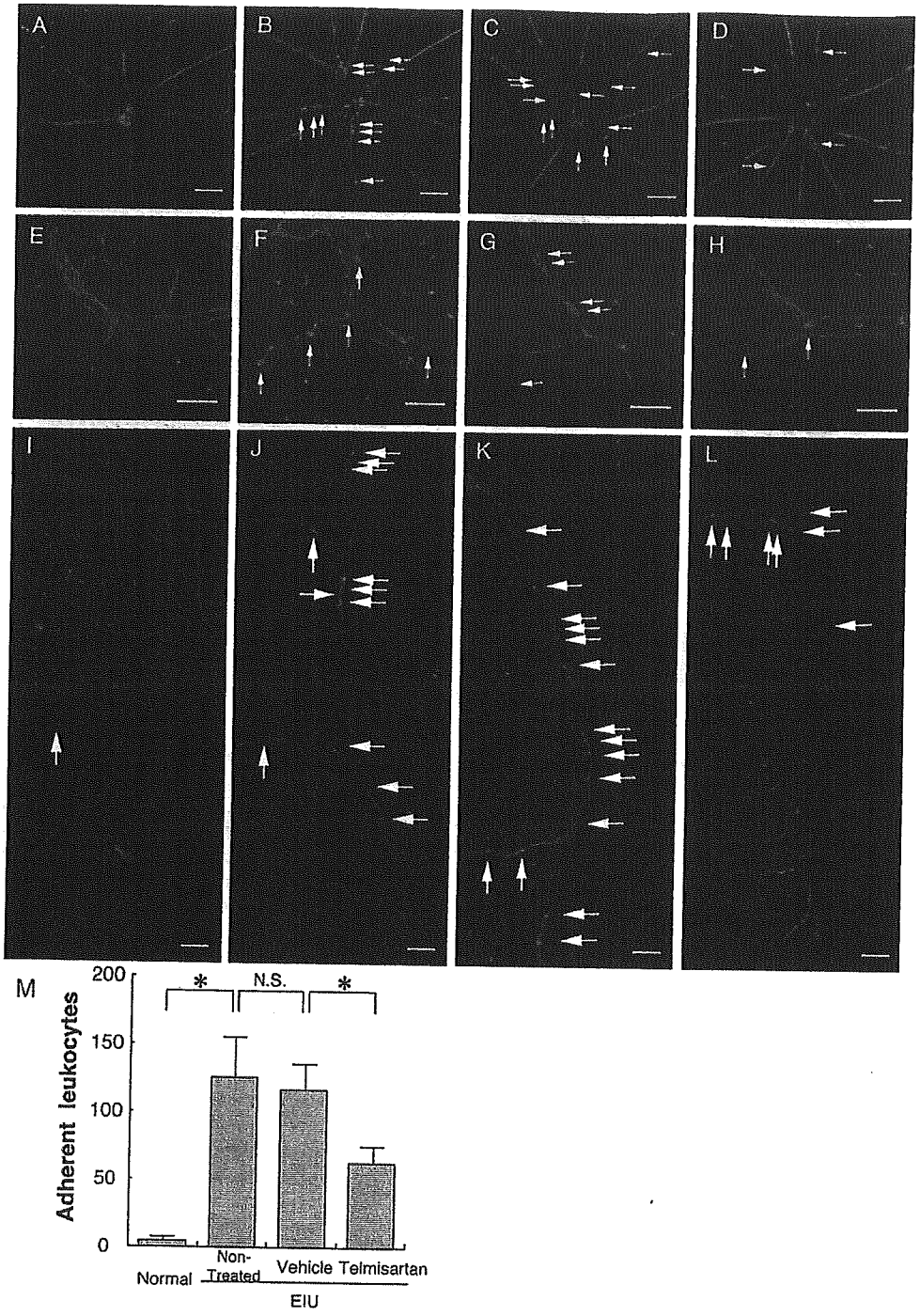

FIGURE 2. Effects of telmisartan on retinal inflammation. Flatmounted retinas from normal mice (**A**, **E**, **I**), nontreated EIU mice (**B**, **F**, **J**), vehicle-treated EIU mice (**C**, **G**, **K**), and telmisartan-treated EIU mice (**D**, **H**, **L**). Nontreated and vehicle-treated EIU mice showing increased adherent leukocytes (*arrows*) compared with normal mice. The treatment with telmisartan decreased the adherent leukocytes. Scale bar, 100 μm. (**M**) Quantification of adherent retinal leukocytes. Telmisartan-treated EIU mice showed significantly fewer adherent leukocytes than did nontreated or vehicle-treated mice. The results represent the mean ± SD; $n = 15$. $^*P < 0.01$ by Mann-Whitney test.

DISCUSSION

The present study demonstrates for the first time that AT1-R upregulation is associated with ocular inflammation in the murine model of EIU and that the AT1-R signaling blockade with telmisartan attenuates several inflammatory parameters including ICAM-1-mediated leukocyte adhesion and infiltration in EIU eyes.

Leukocyte adhesion to the vessel walls is an important process in inflammation. When leukocytes are recruited to inflammatory sites, adhesion molecules play essential roles in the first phase of inflammation. ICAM-1 and its counter receptor β2 (CD18)-integrins (i.e., LFA-1 and Mac-1) regulate the leukocyte-endothelial interaction in the pathogenesis of EIU.[8–10] During the development of EIU, ICAM-1 is upregulated and expressed on vascular endothelial cells of the iris-ciliary body shortly after LPS injection.[9] In addition, several studies have demonstrated that treatment with anti-ICAM-1 antibodies significantly inhibits the development of EIU.[9,10] In the present study, upregulation of retinal ICAM-1 in EIU was suppressed after pretreatment with telmisartan. This finding is supported by recent data from in vitro assays and in vivo models on systemic hypertension and diabetes, showing that AT1-R blockade attenuates ICAM-1 expression.[19,28] Recently, we have demonstrated that administration of telmisartan inhibits pathologic, but not physiological, retinal neovascularization in a murine model of ischemic retinopathy, by prevention of

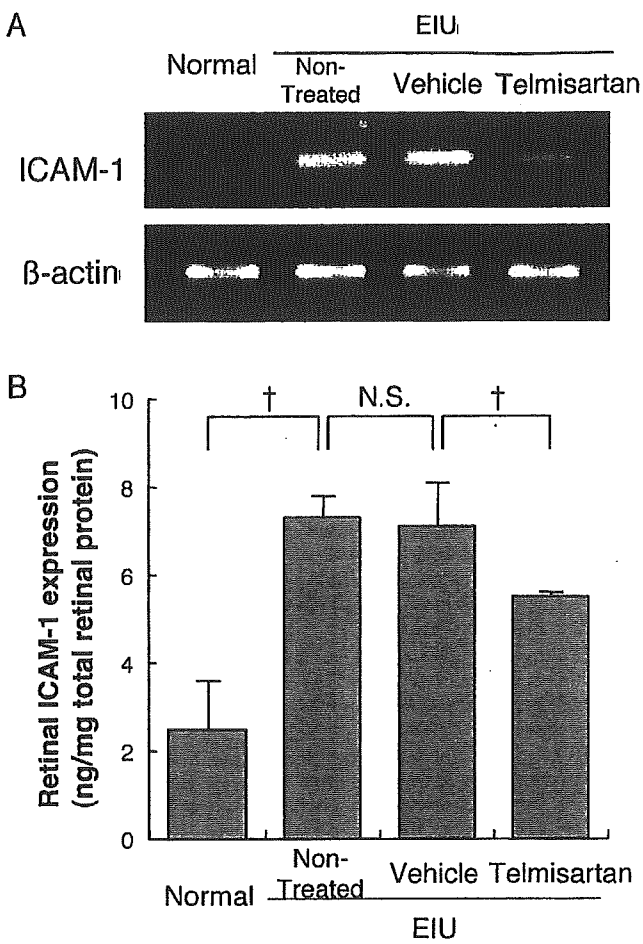

FIGURE 3. Effects of telmisartan on retinal ICAM-1 expression. RT-PCR (A) Retinal ICAM-1 mRNA expression in EIU mice treated with telmisartan was lower than that in nontreated and vehicle-treated EIU mice. Expression was very low in normal mice. (B) Retinal ICAM-1 protein levels in nontreated or vehicle-treated EIU mice were significantly higher than in normal mice and were significantly suppressed by treatment with telmisartan. The results represent the mean ± SD; $n = 6$. †$P < 0.05$ by Mann-Whitney test.

ICAM-1-mediated leukocyte involvement in pathologic neovascularization.[29] The present data on EIU as a model of ocular inflammation more strictly confirm the anti-inflammatory effects of AT1-R blockade in the eye.

Besides ICAM-1, various chemical mediators are involved in the pathogenesis of EIU. In the present study, telmisartan treatment led to the suppression of EIU-induced cytokines including IL-6, TNF-α, COX-2, iNOS, and MCP-1. This result is compatible with those reported previously[30,31] demonstrating the inhibitory effects of AT1-R blockers on these inflammatory cytokines stimulated by LPS in other organs. The proinflammatory effects of angiotensin II are attributable to its induction of these inflammation-related molecules, most of which are downstream products of nuclear factor (NF)-κB, a transcription factor that promotes the gene expression of various inflammatory cytokines.[32] LPS-induced inflammation is mediated by the activation of NF-κB.[32] Indeed, ocular inflammation is suppressed by administration of an NF-κB inhibitor in EIU.[33] Taken together, the evidence shows that the anti-inflammatory effects of AT1-R blockers most likely result from suppressed gene expression of NF-κB-induced molecules. These previous findings, in accordance with our data, suggest that telmisartan affects not only ICAM-1-mediated leukocyte adhesion but also various inflammatory processes.

In the present study, although anterior-chamber cell infiltration was substantially suppressed by telmisartan, little or no significant change was detected in protein leakage. A similar discrepancy between cell infiltration and protein leakage was also noted in several EIU studies by using neutralizing antibodies against ICAM-1, E-selectin,[34] P-selectin,[34] LFA-1,[9,10] and IL-10.[35] Considering that prostaglandin E2, an inflammatory mediator in addition to the adhesion molecules, is operative in protein leakage[36] and that combined inhibition of both L- and P-selectin suppresses protein leakage,[12,34] the cell-protein discrepancy observed in the present and previous studies is most likely attributable to differential mechanisms controlling the multiple inflammatory phases.

Recent reports have revealed that the renin-angiotensin system plays central roles in pathologic vascular conditions including inflammation, angiogenesis, and vascular remodeling.[15–20] The renin-angiotensin system has been shown to exist locally in various organs and to promote inflammation-related pathogenesis in atherosclerosis,[37] cerebral infarction,[38] and pancreatitis.[39] AT1-R blockers other than telmisartan are also reported to be anti-inflammatory.[37–39] These recent findings suggest the possibility of AT1-R blockade as a therapeutic strategy for these disorders characterized by inflammation. In atherosclerosis, in which angiotensin II promotes the infiltration of monocytes and T lymphocytes, AT1-R blockade with irbesartan suppresses the expression of MCP-1 and subsequent macrophage infiltration.[37] In spontaneously hypertensive rats,

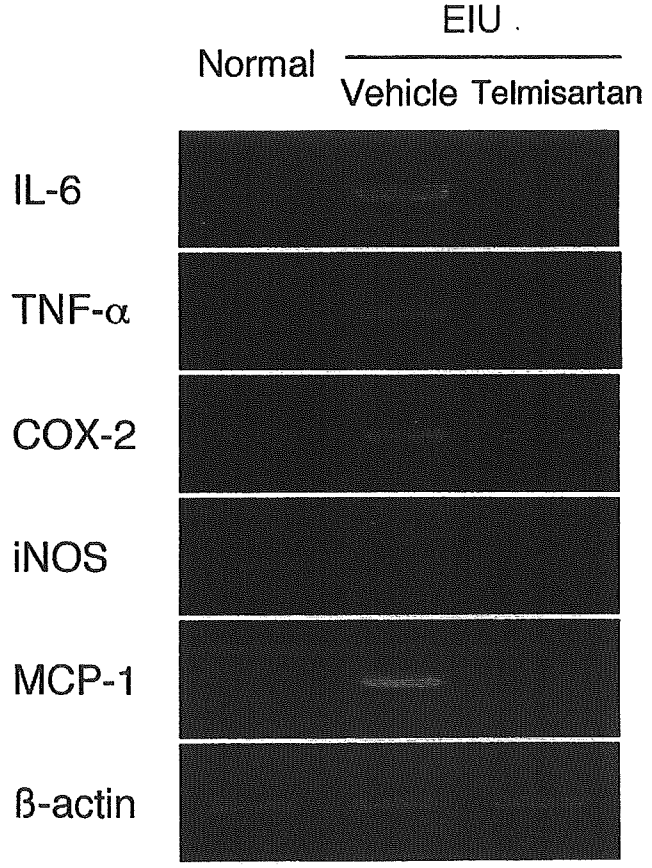

FIGURE 4. Effects of telmisartan on retinal expression of inflammatory mediators. Retinal mRNA expression of IL-6, TNF-α, COX-2, iNOS, and MCP-1 in vehicle-treated EIU mice was higher than in normal, age-matched mice and was suppressed by the administration of telmisartan.

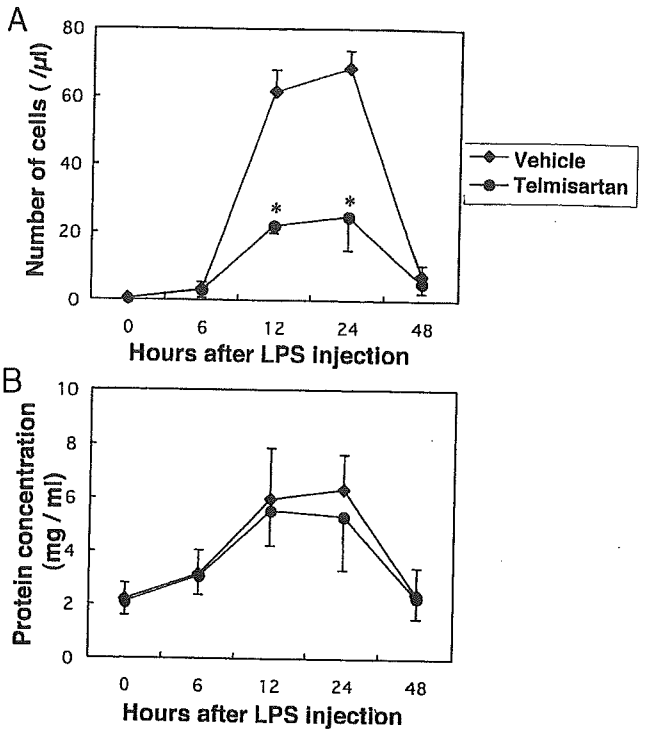

FIGURE 5. Effects of telmisartan on anterior uveitis. (**A**) The number of cells in aqueous humor 12 and 24 hours after LPS injection was markedly reduced by treatment with telmisartan. (**B**) The protein concentration in aqueous humor was not significantly suppressed by the treatment with telmisartan. (♦) Vehicle-treated and (○) telmisartan-treated mice. The results represent the mean ± SD; $n = 15$. $^*P < 0.01$ by Mann-Whitney test.

which are vulnerable to brain ischemia, AT1-R blockade with candesartan suppresses ICAM-1-dependent leukocyte adhesion to the cerebral vessels, protecting against brain ischemia.[38] In acute pancreatitis, AT1-R blockade with losartan suppresses the production of reactive oxygen species by NADPH oxidase and reduces the severity of inflammation.[39] In addition, an angiotensin-converting enzyme inhibitor, widely used as an anti-hypertensive drug, is also reported to suppress vascular inflammation.[40] In the eye, localization of the renin-angiotensin system has been demonstrated without elucidation of its function,[41,42] except the possibility of an intraocular pressure modulator.[42] In the present study, AT1-R mRNA and protein expression is shown to be upregulated during the development of EIU. Further, AT1-R blockade suppressed ICAM-1-mediated leukocyte adhesion and infiltration. These results, in accordance with the previous data on inflammation in other organs, suggest the involvement of the renin-angiotensin system in ocular inflammation.

Currently, ocular inflammation such as chronic endogenous uveitis, is treated mainly with topical and/or systemic application of corticosteroids. During the long-term treatment with corticosteroids, however, care must be taken to guard against both ocular and systemic complications, including cataract, glaucoma, diabetes, hypertension, and osteoporosis. Clinically, AT-1R antagonists are widely and safely used in hypertensive patients. Combined with corticosteroid therapy, the anti-inflammatory effects of AT1-R blockade may benefit patients with chronic uveitis to decrease the rate and degree of the corticosteroid-induced complications. The present study is the first to indicate the potential use of AT1-R antagonists as a novel therapeutic strategy to suppress ocular inflammation.

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Adherence of Intravitreally Injected Triamcinolone Acetonide to the Denuded Retinal Surface After Internal Limiting Membrane Peeling

Internal limiting membrane (ILM) peeling has been performed to treat macular edema, macular hole, and retinal detachment resulting from macular hole.[1] Although some reports have described the ultrastructure of the peeled ILM, the retinal area where the ILM was peeled away is difficult to precisely determine. Peyman et al[2] described a method to visualize the vitreous by intravitreal injection of triamcinolone acetonide as an aid in separation of the posterior hyaloid during pars plana vitrectomy. We describe a case where the area of peeling was clearly delineated by intravitreally injected triamcinolone acetonide.

Case Report

A 54-year-old woman presented to our clinic complaining of visual loss in her left eye. Slit-lamp and ophthalmoscopic examinations disclosed macular edema associated with central retinal vein occlusion. Best-corrected visual acuity was 20/63. Pars plana vitrectomy, ILM peeling, radial optic neurotomy,[3] and intravitreal triamcinolone acetonide injection (4 mg) were carried out. On the following day, triamcinolone acetonide was seen adhering to the retinal site, where the ILM had peeled off, and also to the nasal side of the optic disk, where radial optic neurotomy had been performed (Fig. 1). One week later, most of the triamcinolone acetonide had disappeared, and no pathologic change was apparent (Fig. 2). Macular edema then resolved, and visual acuity recovered to 20/32 after 1 month; this visual acuity was maintained during the 6-month follow-up period.

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Discussion

Studying primate eyes, Nakamura et al[4] reported that with the ILM removed, the inner retina showed an irregular surface on the vitreous aspect. Furthermore, a case report described a dissociated appearance of the optic nerve fiber layer after ILM peeling.[5] These observations explain the findings in the current case. Particles of triamcinolone acetonide presumably adhered to the irregular surface of the portion of the inner retina denuded of ILM, as opposed to the smooth surface of the intact retina. Removal of ILM and radial optic neurotomy are new experimental

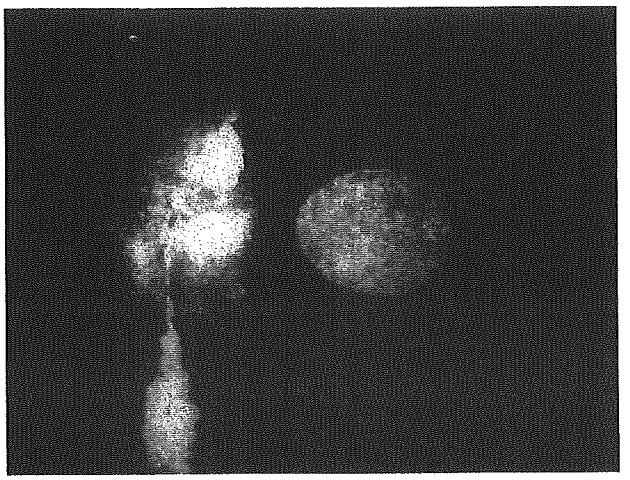

Fig. 1. One day after surgery, white crystals of triamcinolone acetonide adhered to the denuded retinal surface where the internal limiting membrane had been peeled off and to the nasal side of the optic disk where radial optic neurotomy had been performed.

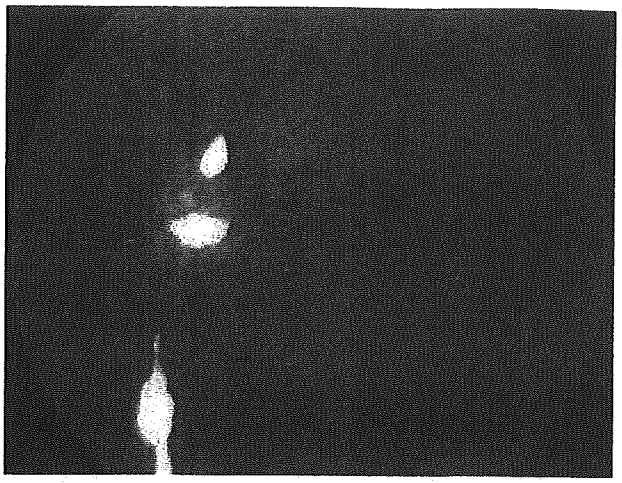

Fig. 2. One week after surgery, most of the triamcinolone acetonide had disappeared, and no adverse effects were apparent.

treatments; thus, further evaluation of these techniques is warranted to prove their efficacy by clinical trials.

Key words: triamcinolone acetonide, internal limiting membrane peeling, radial optic neurotomy.

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Letters to the Editor

Visual Sensations during Pars Plana Vitrectomy under Retrobulbar Anesthesia

Dear Editor:

The visual sensations experienced by patients during cataract surgery have been well documented.[1–3] Here we report the visual sensations described by patients during vitrectomy under retrobulbar anesthesia. Twenty-eight men and 28 women with a mean age of 61.4±12.5 years and a variety of vitreoretinal pathologies (Table 1 [available at http://aaojournal.org]) were studied. The patients were questioned about their visual sensations during and within 3 hours after vitrectomy (Table 2 [available at http://aaojournal.org]). Fifty-four (96.4%) of the patients reported seeing lights, 46 (82.1%) reported seeing ≥1 colors, and 37 (66.1%) reported seeing movements or moving objects. Of the latter 37, 34 saw instruments, and 6 (13.0%) saw the surgeon's fingers or hands. In the 51 cases of triamcinolone-assisted vitrectomy, 24 (47.1%) reported seeing many diffuse whirling black spots. Four patients (7.1%) found the visual experiences frightening. There was no obvious difference in the incidence of the type of sensation for the different vitreoretinal pathologies (Table 3 [available at http://aaojournal.org]). The amount of anesthesia, gender, age, and pain did not seem to affect the number of patients reporting the various visual sensations (Table 4 [available at http://aaojournal.org]). The patients who had lower amounts of anesthesia tended to experience light and color sensations more frequently, but the rates were not statistically significant. A representative patient saw colorless swirling fluid during the early phase of vitrectomy, which probably corresponded to the intraocular irrigation used during core vitrectomy. Later, he reported seeing numerous swirling black and gray spots resembling snowflakes when the white triamcinolone acetonide crystals were injected into the vitreous. Twenty-two patients described a sharply tapered shadow that moved into the center of the field just before the membranelike material was peeled off. The accuracy and precision of the descriptions was unexpected, although we have reported on a patient who not only described but also drew what he saw during vitrectomy with great accuracy.[4] These drawings illustrated how well the visual perception of the patient corresponded with the surgical procedure being performed, even when they were not focused on the retina through the optical system of the eye. The basis for the visual sensations was considered to be similar to that of other entoptic phenomena,[5] as in vitreal floaters in patients with a posterior vitreous detachment. The closer the object to the retina, the more exact will its shadow correspond to the actual shape and size of the object. This explains why some of our patients reported seeing a decrease in the thickness of the object in the center of the visual field (VF) when a rod-shaped instrument was inserted into the vitreous. In addition, patients reported only one instrument on the left side of the VF, and the shaft of the light pipe was not seen. This can be interpreted as the shadow of the instrument but not of the endoillumination probe. It is of some interest that some patients perceived blood as being red; this has been reported previously.[6] Additional investigations are needed to determine the mechanism for this phenomenon. Visual sensations are experienced by the majority of patients despite full pain control, and surgeons should warn patients of these possibilities, as they can be frightening. This should minimize the patients' anxiety and stress during surgery.

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Table 1. Patients' Demographics in Each Vitreoretinal Pathology

	MH (6 Eyes)	ERM (11 Eyes)	ME (19 Eyes)	PDR (9 Eyes)	RRD (8 Eyes)	PVR (2 Eyes)	Macroaneurysm Rupture (1 Eye)
Age (yrs)	68.2±12.9 (53–89)	66±7.2 (55–77)	62±11.6 (30–77)	52.4±12.1 (31–68)	61.1±10.6 (46–74)	41±15.6 (30–52)	85
Gender (male:female)	2:4	4:7	10:9	4:5	6:2	2:0	0:1
Preoperative visual acuity* [range (median)]	0.15–0.7 (0.2)	0.1–1.0 (0.4)	0.06–0.7 (0.2)	HM–0.6 (0.02)	HM–0.8 (0.14)	HM–0.07 (0.0003)	0.1

ERM = epiretinal membrane; HM = hand movements; ME = macular edema; MH = macular hole; PDR = proliferative diabetic retinopathy; PVR = proliferative vitreoretinopathy; RRD = rhegmatogenous retinal detachment.
*Counting fingers and HM were converted to 0.001 and 0.0001, respectively, when the median was calculated according to the previous report (Holladay JT. Visual acuity measurements. J Cataract Refract Surg 2004;30:287–90).

Table 2. Questions

1. Do you see anything?
2. Do you see light?
3. If yes, what color/what shape/what extension is it?
4. Do you see any object?
5. If yes, what does it look like?
6. Did you feel pain? Can you rate the fear you felt as the following scores? 0 = no pain; 1 = weak pain; 2 = moderate pain; 3 = terrible pain.
7. Did you feel that your visual experience was frightening?
8. Considering your visual experience, now which do you think is better: to have the same operation under local anesthesia, or under general anesthesia?

Table 3. Frequency of Various Visual Sensations

	MH (6 Eyes)	ERM (11 Eyes)	ME (19 Eyes)	PDR (9 Eyes)	RRD (8 Eyes)	PVR (2 Eyes)	Macroaneurysm Rupture (1 Eye)
Light	6 (100)	11 (100)	19 (100)	7 (77.8)	8 (100)	2 (100)	1 (100)
Color	5 (83.3)	9 (81.8)	15 (78.9)	7 (77.8)	8 (100)	1/2 (50)	1 (100)
Moving object							
Instrument	5 (83.3)	7 (63.6)	14 (73.7)	6 (66.7)	2 (25)	0 (0)	0 (0)
Triamcinolone acetonide particles	5 (83.3)	0/6 (0)*	11 (57.9)	4 (44.4)	2 (25)	0 (0)	1 (100)
Vitreous	6 (0)	0 (0)	2 (18.2)	3/7 (42.8)*	0 (0)	0 (0)	0 (0)
Membranes	4 (66.7)	4 (36.4)	3/15 (20)*	0 (0)	0 (0)	0 (0)	0 (0)
Other	3 (50)	0 (0)	4 (21.1)	2 (22.2)	2 (25)	0 (0)	1 (100)
Frightening	0 (0)	1 (9.1)	2 (18.2)	1 (11.1)	0 (0)	0 (0)	0 (0)

ERM = epiretinal membrane; HM = hand movements; ME = macular edema; MH = macular hole; PDR = proliferative diabetic retinopathy; PVR = proliferative vitreoretinopathy; RRD = rhegmatogenous retinal detachment.
Data are shown as no. of eyes (%), except for some values indicated by asterisks, which reflect that the procedure was performed only in selected eyes (the denominator shows the no. of eyes).

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CASE REPORT

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Massive bilateral vitreoretinal hemorrhage in patient with chronic refractory idiopathic thrombocytopenic purpura

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Abstract *Background:* We describe a patient with chronic, refractory idiopathic thrombocytopenic purpura (ITP) who developed massive vitreoretinal hemorrhages in both eyes. *Methods:* A 49-year-old woman complained of an acute decrease of vision, and ophthalmoscopy revealed bilateral preretinal and subretinal hemorrhages around the optic disc. Her platelet counts were less than 5000/μl in spite of repetitive administration of corticosteroids and immunoglobulins with splenectomy for the chronic refractory ITP. One month later, she developed massive vitreoretinal hemorrhages bilaterally, and vision decreased to hand-motion. *Results:* Vitrectomy was performed on both eyes after medical treatment to increase platelet counts. The intraretinal hemorrhages were absorbed from the area around the disc and vision recovered to 20/100 in the right eye and 20/2000 in the left after one month. *Conclusion:* Massive vitreoretinal hemorrhages can develop in patients with chronic refractory ITP, and vitrectomy can be beneficial for this condition.

Keywords Idiopatic thrombocytopenic purpura · Subretinal hemorrhage · Thrombocytopenia · Vitrectomy · Vitreous hemorrhage

Introduction

Idiopathic thrombocytopenic purpura (ITP) is characterized by refractory thrombocytopenia, production of autoantibodies, and persistent predisposition to bleeding affecting virtually all mucocutaneous tissues and various organs [1, 2, 4, 5, 7]. The common clinical manifestations include subcutaneous and mucosal bleeding, and ocular complications such as subcutaneous hemorrhages in the eyelids and subconjunctival hemorrhages [6]. Occasional intraretinal dot hemorrhages have been reported [4, 5, 7]. To the best of our knowledge, ocular complications that lead to severe visual impairments have rarely been described in patients with ITP. We report a patient with chronic refractory ITP who developed massive vitreoretinal hemorrhages bilaterally, resulting in severe vision depression.

Case report

A 49-year-old Japanese woman with ITP was referred to the ophthalmology ward of Keio University Hospital for ocular complications in Feburary, 2004. Her visual acuity was 20/20 OU. Ophthalmoscopy showed multiple dot hemorrhages bilaterally with no remarkable findings in the anterior segments. The patient was severely thrombocytopenic with a platelet count of <2000/μl. She had mucocutaneous bleedings and pathognomonic menorrhagia accompanied by severe thrombocytopenia at age 30 years. Repetitive treatments with high-dose intravenous corticosteroids and immunoglobulins with splenectomy, resulted in only a transient rise in the platelet count. We thus confirmed her diagnosis of chronic refractory ITP.

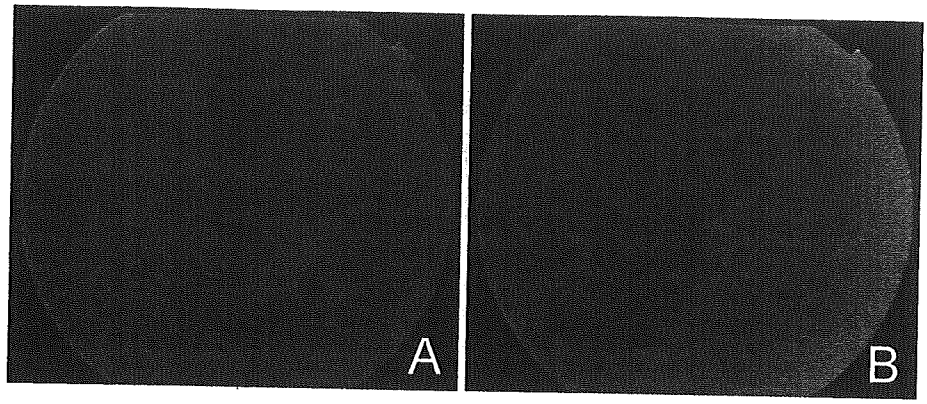

Fig. 1 Fundus photograph of the right eye (**A**) and the left eye (**B**). Bilateral isolated preretinal and intraretinal hemorrhages with small macular hemorrhages are seen

Her visual acuity had decreased to 20/300 OD and 20/200 OS in June, 2004. Funduscopic examination revealed bilateral preretinal and intraretinal hemorrhages around the optic disc discontinuous with small macular hemorrhage (Fig. 1). A month later, her visual acuities were barely hand-motion OU, and massive vitreous hemorrhages were ob-

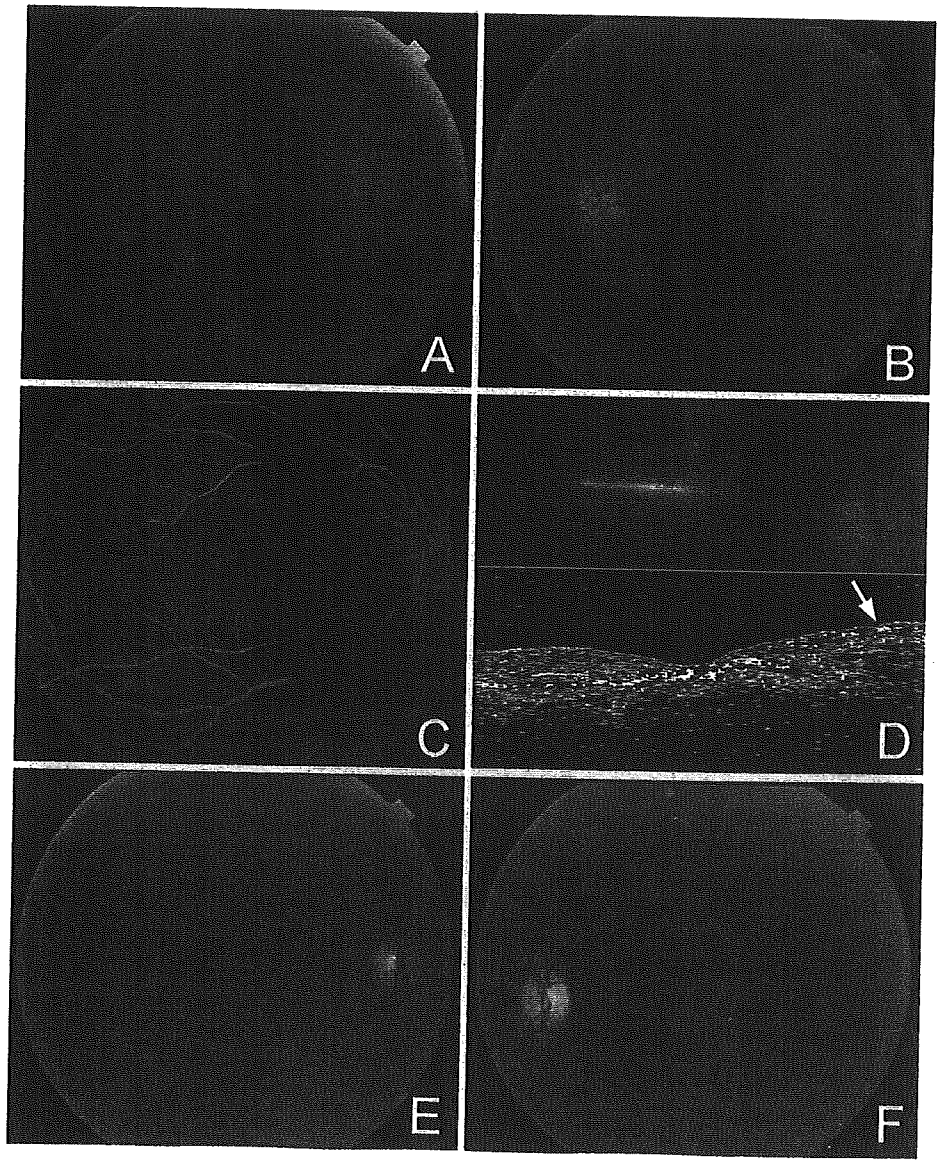

Fig. 2 Postoperative fundus photographs, fluorescein angiogram and optical coherence tomographic images. Fundus photograph of the right eye (**A**) and the left eye (**B**) 1 month after vitrectomy, showing partial absorption of the intraretinal hemorrhages around the optic disc. **C** Fluorescein angiogram of the right eye showing a blockage by residual intraretinal and subretinal hemorrhages and dilation of the peripapillary vessels. **D** Horizontal optical coherence tomographic scan showing a highly reflected area in the inner retina (arrow) and the outer retina at the macula, indicating a persistent intraretinal hemorrhage and submacular hemorrhage. Fundus photographs of the right eye (**E**) and the left eye (**F**) 4 months after the surgery showing minimal damage to the retinal pigment epithelium at the macula in the right eye and submacular fibrosis in the left eye

served in both eyes. The hemorrhages prevented a detailed examination of the fundus. Ultrasound echography detected the presence of a posterior vitreous detachment, but no findings concerning the source or location of the hemorrhage were found.

Her platelet count was <3000/μl and hemoglobin concentration was 8.4 mg/dl, but blood clotting was within normal, prothrombin time (%PT); 94% and activated partial thromboplastin time (APTT); 25.8 s. The patient was placed on high-dose oral corticosteroids and intravenous immunoglobulins together with daily transfusions of concentrated platelets for surgical treatment to recover her vision. The platelet count was successfully restored to about 30,000/μl. Vitrectomy was recommended, and after discussing the purpose and procedures of vitrectomy, signed informed consent was obtained.

Results

Vitrectomy was then perfomed on the left eye on August 31, 2004 and on the right eye on the following day. After the massive vitreous hemorrhage was removed, multiple foci of preretinal hemorrhages and diffuse subretinal hemorrhages involving the macula were found. The subretinal hemorrhages were not removed because of the difficulty in removing old yellowish blood clots, and her tendency to bleed.

Her visual acuities recovered to 20/100 OD and 20/2000 OS after 1 month with absorption of the retinal hemorrhages especially the intraretinal hemorrhages around the optic disc, but the subretinal hemorrhages had expanded (Fig. 2A, B). Fluorescein angiography showed a blockage of the choroidal pattern by residual intraretinal and subretinal hemorrhages. The peripapillary vessels were dilated (Fig. 2C). A horizontal optical coherence tomograpic (OCT) scan showed a highly reflected area in the inner retina, the absence of the deeper reflecting band, and a highly reflected area in the outer retina at the macula. These findings indicated a persistent intraretinal hemorrhage and submacular hemorrhage (Fig. 2D). The intraretinal hemorrhage was completely absorbed with minimal damage to the retinal pigment epithelium at the macula in the right eye and submacular fibrosis in the left eye after 4 months. The vision was maintained at 20/100 OD and 20/2000 OS (Fig. 2E, F) and no ischemic change was observed in optic nerve such as initial sign of anterior ischemic neuropathy until her last postoperative visit at 7 months. Peripheral visual field was normal except for residual central scotoma.

Discussion

Massive subretinal hemorrhages have been described in a patient with age-related macular degeneration (ARMD) with ITP [3]. However, our patient did not have a history of ARMD or choroidal neovascularization by funduscopic examination performed before the vitreous hemorrhage and fluorescein angiography after vitrectomy. Rubenstein et al. reported a high incidence of retinal hemorrhages in patients with severe anemia of hemoglobin concentration (<8 mg/dl) and severe thrombocytopenia (<50,000/μl), but a much lower frequency in anemic patients without thrombocytopenia [4]. The retinal hemorrhages were generally flame-shaped, but were occasionally either subhyaloid or had broken through the internal limitting membrane of the retina. Bilateral round macular hemorrhage with flame-shaped hemorrhages were described in two ITP patients with severe thrombocytopenia (<20,000/μl), one with myelofibrosis and the other with acute lymphocytic leukemia [4].

Our patient had a more severe thrombocytopenia (<3000/μl) with moderate anemia when she developed massive vitreoretinal hemorrhages. Numerous intraretinal hemorrhages, along with bilateral intraretinal hemorrhages in the macular areas, predominantly in the outer plexiform layer, were described in a patient with myelofibrosis [4]. The dilation of the peripapillary vessels and persistent intraretinal hemorrhages detected by OCT in our patient indicated that the vitreous hemorrhages might have developed from an extension of the intraretinal hemorrhage by disruption of the peripapillary vessels. However, the submacular hemorrhages, which were detected before the development of the vitreous hemorrhage, might have developed through disruption of the deeper retinal vessels in the macula such as in the outer plexiform layer. The initial development of the macular hemorrhages might be the cause of her limited visual improvement after complete absorpsion of the intraretinal hemorrhages and vitreous hemorrhages by successful vitreous surgery.

Although systemic cutaneous purpura and nasobuccal hemorrhages occur in all patients with ITP, the location of the hemorrhages are varied with preservation of certain vital organs such as the brain, heart, and eyes [1–3, 7]. The pathogenetic mechanisms that determine the propensity and degree of thrombocytopenic hemorrhages have not been determined. Thus, the complex associations of the coagulative state, vascular endothelium integrity, capillary permeability, inflammatory processes, cytokines, leakiness and/or extravasation of blood components remain to be determined in ITP.

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LABORATORY INVESTIGATION

Effect of Topical Unoprostone Isopropyl on Optic Nerve Head Circulation in Controls and in Normal-Tension Glaucoma Patients

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Abstract

Purpose: To evaluate the effect of unoprostone isopropyl on microcirculation in the optic nerve head (ONH) of controls and patients with normal-tension glaucoma (NTG).

Methods: Thirty healthy volunteers were randomly placed in a placebo group or a control group. For ten NTG patients, one eye was selected to receive the placebo drops and the contralateral eye received the unoprostone in a masked fashion. In both studies, the intraocular pressure (IOP) and the parameters of the blood hemodynamics of the ONH were obtained before and at 1 and 2 h after the instillation. Blood flow measurements were made with a scanning laser Doppler flowmeter.

Results: In both control subjects and NTG patients, the changes in the IOPs after the instillation of either unoprostone or the placebo were not significant because almost all of the NTG patients had IOPs lower than 15 mmHg. Although the hemodynamic parameters were not significantly changed in the placebo-treated eyes of the controls, the eyes of the controls treated with unoprostone had mean blood velocity and flow values that were significantly higher than the baseline values 1 and 2 h after instillation ($P < 0.01$). The velocity values of the controls treated with unoprostone were significantly higher than in those controls receiving the placebo at 2 h postinstillation ($P = 0.027$). The values for the three circulation parameters (volume, velocity, flow) were significantly higher than the baseline values after instillation in the eyes of the NTG patients treated with unoprostone ($P < 0.05$). In contrast, none of these parameters was significantly different from the baseline in the eyes of NTG patients treated with placebo.

Conclusions: These results showed that unoprostone significantly increased microcirculation in the ONH in control subjects and in NTG patients without reducing the IOP significantly. **Jpn J Ophthalmol** 2005;49:287–293 © Japanese Ophthalmological Society 2005

Key Words: human study, normal-tension glaucoma, ocular blood flow, optic nerve head circulation, unoprostone isopropyl

Introduction

Unoprostone isopropyl is a chemically synthesized docosanoid (22-carbon basic skeleton) that has been extensively evaluated by laboratory[1–3] and clinical[4–14] studies. The results of a clinical trial suggested that twice daily topical administration of 0.12% unoprostone was comparable to twice daily administration of 0.5% timolol for lowering the intraocular pressure (IOP) in eyes with primary open-angle glaucoma (POAG) or chronic angle-closure glaucoma and in eyes with ocular hypertension (OH).[5,11,12] Timolol maleate 0.5% and unoprostone 0.15% therapy given twice daily were comparable to a timolol 0.5%/dorzolamide 2% fixed combination in lowering the IOP of patients with POAG and OH.[13] Unoprostone 0.15% as an adjunctive therapy to timolol was also comparable to brimonidine 0.2% and dorzolamide 2.0%.[14] Studies have also shown that unoprostone had no effects on cardiovascular functions.[5–7]

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Although the reduction of the IOP has been considered to be important in controlling many types of glaucoma, the multifactorial nature of this disease is becoming more and more recognized. Several reports have shown that visual field deterioration can be observed despite satisfactory control of IOP.[15–19] Vascular changes in the optic nerve head (ONH) may be involved in the pathogenesis of the glaucomatous changes and, in particular, in the pathogenesis in eyes with normal-tension glaucoma (NTG).[20–24]

We investigated the effects of topically applied unoprostone on microcirculation in the ONH by measuring the capillary blood flow in the neuroretinal rim area using a scanning laser Doppler flowmeter (SLDF).

Materials and Methods

The procedures of both study 1 and study 2 conformed to the guidelines of the Declaration of Helsinki, and informed consent was obtained before the measurements from all patients and controls. The IOP and blood flow measurements in the ONH of each subject were performed before eyedrop instillation (baseline) and at 1 and 2h after the instillation.

Blood flow measurements were made with the SLDF using a Heidelberg retinal flowmeter (HRF; Heidelberg Engineering, Heidelberg, Germany). The principle, validity, and reliability of the SLDF in measuring ocular blood flow have been previously described.[25,26] The SLDF data were collected from a 2.7mm × 0.7mm area of the rim area of the optic disc, and 256 points on 64 lines were obtained with a measurement accuracy of 10μm (per point). The scanning beam had a wavelength of 795nm, a power of 100μW, and a data acquisition time of 2.048s. Every line was scanned 128 times at a line-sampling rate of 4000Hz. By performing a discrete, fast Fourier transformation on the 128 scans of each point, a power spectrum was obtained and the independent variables of volume and flow were calculated. Velocity was then calculated by dividing the flow values by the volume values.

The pupil was not dilated, and subjects were asked to fixate on a distant target during the examination. Several mapped images were then acquired from the superior area of the optic disc. In each subject, three good-quality images that were suitable for blood flow measurements of the neuroretinal rim area were selected and analyzed. Poor-quality images, mainly due to gross eye movements and poor fixation, were excluded from the analyses. The hemodynamic variables mean blood volume (volume), mean blood velocity (velocity), and mean blood flow (flow) were analyzed in the superotemporal neuroretinal rim area, avoiding ophthalmoscopically visible vessels (Fig. 1). The analysis

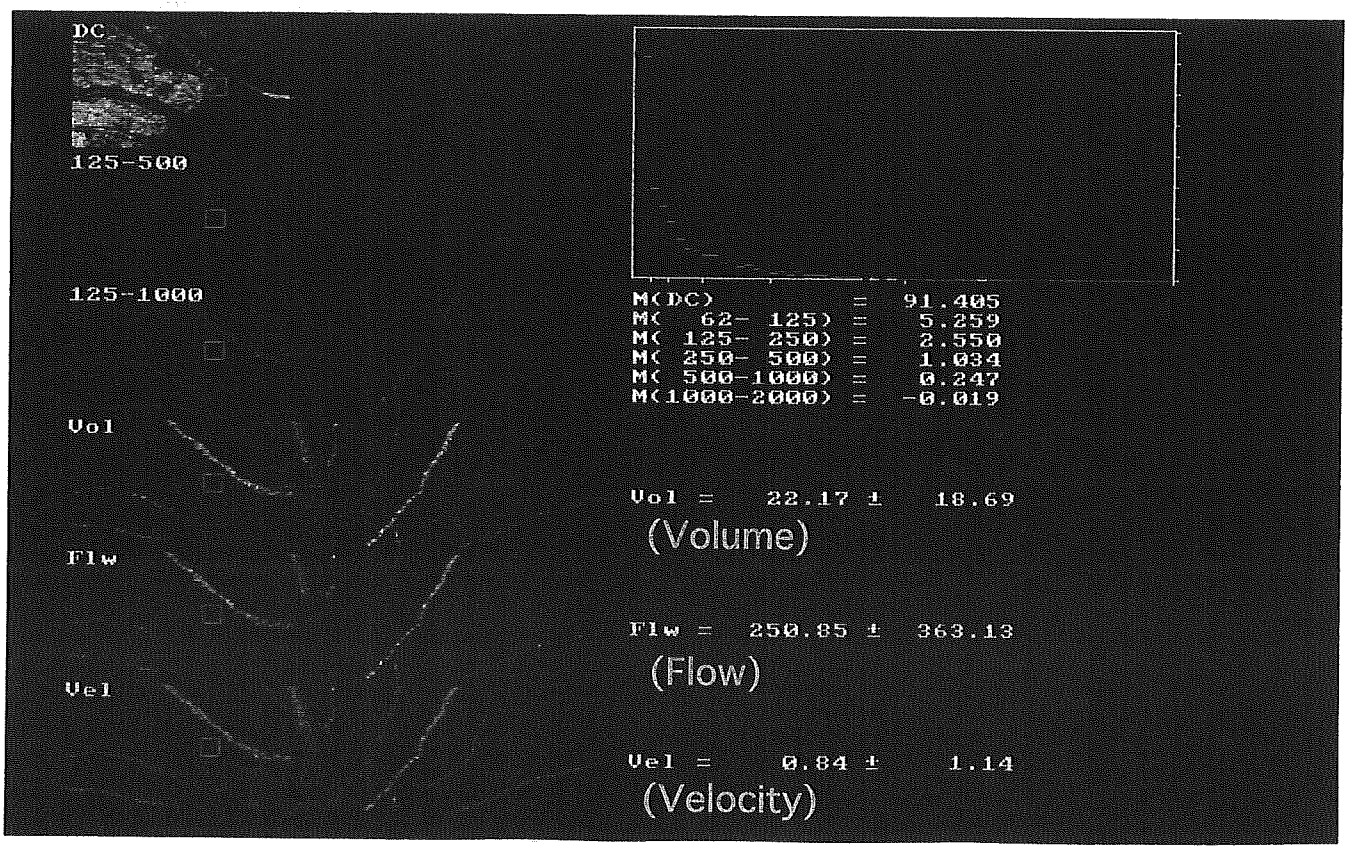

Figure 1. Perfusion map in control subjects. Measurement area (10 × 10 pixels) at superotemporal rim area (*arrow*).

Table 1. Backgrounds of the two groups in Study 1

	Sex	Mean age (years)	Mean refraction (diopters)
Control group	4 men & 11 women	24.0 ± 3.4	−3.27 ± 2.2
Isopropyl unoprostone-treated group	3 men & 12 women	23.5 ± 4.8	−3.36 ± 2.3

The mean age and mean refraction represent the average ± SD. There were no statistically significant differences in the backgrounds of the two subject groups.

window was 10 × 10 pixels (100 × 100 μm), and, as much as possible, the same area was measured in each session.

Bottles of the active drug and placebo were provided with coded labels so that neither the investigators nor the subjects had any knowledge of the substance being administered, and the same investigator (KI) obtained the SLDF images and analyzed the data of ocular microcirculation for all subjects in a masked fashion.

Study 1. Blood Flow Changes in Control Subjects

All control subjects underwent a thorough ophthalmologic examination, including measurements of visual acuity and IOP, slit-lamp examination, and indirect funduscopy. The exclusion criteria were the presence of any ophthalmologic disease in the anterior or posterior segment of the eye or any systemic abnormalities. Subjects were also excluded if they had undergone intraocular or laser surgery, or had taken any medications within the 2 weeks prior to the study. Seven men and 23 women met the criteria, and they were randomized to each treatment group (Table 1). There were no statistically significant differences in the backgrounds of the two subject groups.

After the IOPs were measured by applanation tonometry, the subjects received 30 μl of either 0.12% unoprostone (Fujisawa Pharmaceutical, Osaka, Japan) or placebo in the right eye. The placebo consisted of the unoprostone vehicle solution.

Study 2. Blood Flow Changes in NTG Patients

Ten NTG patients, aged 30 to 77 years (53.7 ± 16.47 years, mean ± SD), were enrolled in the randomized, placebo-eye-controlled (contralateral eye) double-masked study. They comprised six men and four women, and all were diagnosed as having NTG after, as inpatients, their IOP had been monitored every 3 h for 24 h. Ophthalmological examinations also showed that they had normal open angles, glaucomatous ONH cupping, and corresponding visual field defects. The IOP values, determined by applanation tonometry, were within the normal range (<21 mmHg). The exclusion criteria for the NTG patients were ophthalmic diseases such as uveitis, ocular trauma, or any other ocular pathologic findings observed during the slit-lamp and indirect funduscopic examination except for the glaucomatous damage. Brain magnetic resonance imaging was performed to rule out cerebral diseases in all patients. In addition, patients with any history of surgery, systemic hypertension, or cardiovascular disease were excluded. Patients being treated with corticosteroids, β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, platelet active agents, or carbonic anhydrase inhibitors were also excluded. A 4-week washout period preceded the beginning of the study for patients taking any topical medications.

Patients received instillation of 30 μl of unoprostone (0.12%) into one randomly selected eye while the contralateral eye received the placebo.

Statistical Methods

Three selected SLDF images were analyzed by the masked investigator, and the averages of the measurements from the three images were used for statistical analyses. For sample size calculations, we assumed a standard deviation of 20% of the mean difference between repeated measurements obtained with the HRF,[27] a type I error of 0.05, and a power to detect a difference of 20% between sessions[28] of 80%. We calculated that a sample size of ten individuals would meet these criteria.

To evaluate the relationship between unoprostone-treated eyes and placebo-treated eyes, the ratio of each measurement value to the baseline value of the blood flow parameters and IOPs were evaluated by the Friedman test followed by Fisher's protected least significant difference (PLSD) post hoc test. The ratios of each hemodynamic parameter between unoprostone-treated eyes and placebo-treated eyes for each study were compared by analysis of variance (ANOVA) followed by Fisher's PLSD. The ratios of each hemodynamic parameter between unoprostone-treated eyes in study 1 and those in study 2, that is, normal subjects versus NTG patients, were compared in the same manner. A level of $P < 0.05$ was accepted as statistically significant.

Results

Study 1. Blood Flow Changes in Control Subjects

The values of the IOPs obtained in control subjects treated with unoprostone or placebo are listed in Table 2. The changes in the IOPs were not statistically different from the

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Table 2. Intraocular pressure (IOP) changes in Study 1

	Preinstillation	1 h after instillation	2 h after instillation
Control group	11.8 ± 2.3	11.3 ± 2.1	11.5 ± 1.8 (mmHg)
Isopropyl unoprostone-treated group	12.1 ± 1.8	11.1 ± 2.4	11.5 ± 2.0 (mmHg)

The IOP values represent the average ± SD. There were no statistically significant differences after instillation of either unoprostone or placebo compared with baseline values.

Table 3. IOP changes in Study 2

	Preinstillation	1 h after instillation	2 h after instillation
Placebo-treated eyes	11.9 ± 2.6	12.0 ± 3.0	11.9 ± 2.6 (mmHg)
Isopropyl unoprostone-treated eyes	13.1 ± 2.7	12.8 ± 2.8	12.0 ± 3.2 (mmHg)

The IOP values represent the average ± SD. There were no statistically significant differences after instillation of either unoprostone or placebo compared with baseline values.

baseline values after instillation of either unoprostone or placebo.

Two hours after the instillation of unoprostone, volume increased by 5.7% over the baseline values, but this increase was not statistically significant ($P = 0.118$) (Fig. 2A). Velocity in eyes treated with unoprostone increased by 11.1% at 1 h after instillation ($P = 0.0059$), and by 16.2% at 2 h after instillation ($P = 0.001$) (Fig. 2B). Flow in eyes treated with unoprostone increased by 11.6% at 1 h after instillation ($P = 0.0041$), and by 14.7% at 2 h after instillation ($P = 0.0004$) over the baseline values (Fig. 2C).

In contrast, there were no significant changes in volume, velocity, or flow compared with the baseline values in eyes receiving placebo ($P > 0.05$).

A comparison of volume, velocity, and flow values between normal subjects treated with unoprostone versus those receiving placebo showed that velocity was significantly higher in the unoprostone-treated eyes 2 h after the instillation ($P = 0.0266$).

Study 2. Blood Flow Changes in NTG Patients

The IOPs for the placebo- and unoprostone-treated eyes at each time point are listed in Table 3. Compared with the baseline values, there were no statistically significant changes in the IOP values after instillation of either unoprostone or placebo.

Two hours after instillation of unoprostone, volume increased by 9.6% over the baseline values ($P = 0.0282$) (Fig. 3A). In addition, velocity in eyes treated with unoprostone increased by 17.3% at 2 h after instillation ($P = 0.0289$) (Fig. 3B). Flow in eyes treated with unoprostone increased by 18.2% over the baseline values ($P = 0.025$) at 2 h after the instillation (Fig. 3C).

In contrast, there were no significant changes in volume, velocity, or flow compared with the baseline values in eyes receiving placebo ($P > 0.05$).

The three parameters of blood flow in NTG eyes treated with unoprostone were not significantly different from those in NTG eyes receiving placebo at 1 or 2 h postinstillation ($P > 0.05$).

Discussion

Our randomized, double-masked, placebo-controlled study demonstrated that topical unoprostone significantly increased the microcirculation in the neuroretinal rim area of the ONH in healthy volunteers and NTG patients. In addition, our results demonstrated that the improved ocular blood flow occurred without a significant reduction of IOP.

Unoprostone has been shown in several studies to lower the IOP in patients with ocular hypertension, primary open-angle glaucoma, and chronic angle-closure glaucoma.[5,11–14] In this study, the participants were healthy volunteers and NTG patients, and almost all of the NTG patients had IOPs lower than 15 mmHg. Thus, it is not surprising that we did not see a significant IOP lowering effect.

In retrospect, it would have been useful to have measured the blood pressure and pulse rate in the participants, as these parameters could have affected the perfusion pressure and, thus, blood flow values. However, it has been reported that unoprostone has no effect on cardiovascular functions.[5–7] These results suggest that unoprostone increased the ocular blood flow independently of its effect on the perfusion pressure. Furthermore, at 2 h after instillation, the increase in tissue microcirculation tended to be better in NTG patients receiving topical unoprostone than in the controls.