TABLE 2. Risk Factors for Elevated Intraocular Pressure Elevation of ≥ 24 mm Hg After Triamcinolone Acetonide Treatment—Cox Proportional Hazards Analysis

Variable	Hazards Ratio for ≥ 24 mm Hg	*P* Value
Model 1: All eyes treated with triamcinolone acetonide injection (n = 427)		
Age (years)	0.96 (0.95 to 0.98)	<.0001*
Diabetes mellitus	0.76 (0.55 to 1.02)	.068
IVI included	1.89 (1.41 to 2.52)	<.0001*
IOP at baseline (mm Hg)	1.15 (1.05 to 1.27)	.003*
Model 2: Eyes with STI only (n = 333)		
Age (year)	0.96 (0.94 to 0.99)	.003*
Diabetes mellitus	0.91 (0.60 to 1.38)	.647
STI (mg)	1.07 (1.03 to 1.12)	.0006*
IOP at baseline (mm Hg)	1.31 (1.13–1.52)	.0003*
Model 3: Eyes with IVI only (n = 57)		
Age (year)	0.98 (0.94 to 1.03)	.393
Diabetes mellitus	0.91 (0.47 to 1.61)	.760
IVI (mg)	1.64 (1.09 to 3.39)	.013*
IOP at baseline (mm Hg)	1.03 (0.85 to 1.27)	.765
Model 4: Eyes with 20 mg of STI or 20 mg of STI plus 4 mg of IVI (n = 201)		
Age (year)	0.95 (0.92 to 0.98)	.003*
Diabetes mellitus	0.75 (0.41 to 1.29)	.306
Plus 4 mg of IVI	2.27 (1.33 to 4.02)	.003*
IOP at baseline (mm Hg)	1.28 (1.07 to 1.55)	.008*

IOP = intraocular pressure; IVI = intravitreal injection of triamcinolone acetonide; STI = sub-Tenon capsule injection of triamcinolone acetonide.
Hazards ratio is shown with 95% confidence interval.
*$P < .05$.

(11.7%) of the 427 eyes had an elevated IOP of 24 mm Hg or higher. IOP elevation of 24 mm Hg or above started 0.5 month after the injection in 12 eyes, after one month in nine eyes, after two months in 19 eyes, after three months in nine eyes, and after six months in one eye. Patient data before TA injection for the group with IOP elevation of less than 24 mm Hg and the group with 24 mm Hg or T1 higher are shown in Table 1. The patients within the 24 mm Hg or higher group were younger, were less likely to have a history of diabetes mellitus, had a greater incidence of IVI administration of TA, and had higher baseline IOP values. The multivariate Cox proportional hazards regression showed that younger age (HR, 0.96 per year; 95% confidence interval [CI], 0.95 to 0.98; $P < .0001$), the inclusion of IVI (HR, 1.89; 95% CI, 1.41 to 2.52; $P < .0001$), and higher baseline IOP (HR, 1.15 per mm Hg; 95% CI, 1.05 to 1.27; $P = .003$) were risk factors for IOP T2 elevation (Table 2; Model 1).

We also examined whether IOP elevation after TA injection was dose-dependent. In eyes treated by STI (n = 333), one of 36 eyes (2.8%), six of 164 eyes (3.7%), and 18 of 133 eyes (13.5%) showed IOP values of 24 mm Hg or higher after doses of 12 mg, 20 mg, and 40 mg by STI, respectively. Cox proportional hazards regression analysis of the 333 eyes identified younger age (HR, 0.96 per year; 95% CI, 0.94 to 0.99; $P = .003$), a higher dose administered by STI (HR, 1.07 per mg; 95% CI, 1.03 to 1.12; $P = .0006$), and higher baseline IOP (HR, 1.31 per mm Hg; 95% CI, 1.13 to 1.52; $P = .0003$) as risk factors (Table 2; Model 2). In eyes treated by IVI (n = 57), one of 18 eyes (5.6%) and 14 of 39 eyes (35.9%) were associated with IOP of 24 mm Hg or higher after doses of 4 mg and 8 mg by IVI, respectively. Cox proportional hazards regression analysis of the 57 eyes showed that a higher dose administered by IVI (HR, 1.64 per mg; 95% CI, 1.09 to 3.39; $P = .013$) was a risk factor. However, neither younger age (HR, 0.98 per year; 95% CI, 0.94 to 1.03; $P = .393$) nor higher baseline IOP (HR, 1.03 per mm Hg; 95% CI, 0.85 to 1.27; $P = .765$) were significant risk factors (Table 2; Model 3). Additionally, 10 of 37 eyes (27.0%) were associated with an IOP of 24 mm Hg or higher after simultaneous administration by STI (20 mg) and IVI (4 mg). In eyes treated with 20 mg by STI, or with both 20 mg by STI and 4 mg by IVI (n = 201), younger age (HR, 0.95 per year; 95% CI, 0.92 to 0.98; $P = .003$), the addition of 4 mg by IVI (HR, 2.27, 95% CI, 1.33 to 4.02; $P = .003$), and baseline IOP (HR, 1.28 per mm Hg; 95% CI, 1.07 to 1.55; $P = .008$) were identified as risk factors (Table 2; Model 4).

TABLE 3. Data Before the Second Triamcinolone Acetonide Treatment in < 24 mm Hg and ≥ 24 mm Hg Groups

Characteristic (n = 108)	Eyes of < 24 mm Hg (n = 92) n (%)	Eyes of ≥ 24 mm Hg (n = 16) n (%)	*P* Value
Male gender	53 (57.6)	10 (62.5)	.714
Mean age (years)	63.1 ± 11.0	55.6 ± 17.8	.279
Diabetes mellitus	50 (54.3)	8 (50.0)	.748
Hypertension	42 (45.7)	3 (18.8)	.044*
Cataract surgery	33 (35.9)	6 (37.5)	.900
Vitrectomy	24 (26.1)	5 (31.3)	.667
IVI included	4 (4.3)	4 (25.0)	.004*
Mean IOP at baseline (mm Hg)	13.8 ± 3.0	13.5 ± 2.4	.900
ΔIOP after the 1st injection (mm Hg)	3.0 ± 3.2	5.8 ± 2.1	<.0001*
Interval between 1st and 2nd injections (months)	5.2 ± 3.2	5.1 ± 3.2	.841

IOP = intraocular pressure; IVI = intravitreal injection of triamcinolone acetonide; ΔIOP = maximal IOP minus baseline IOP.
*$P < .05$.

TABLE 4. Risk Factors for Elevated Intraocular Pressure of ≥ 24 mm Hg After Second Triamcinolone Acetonide Injection—Cox Proportional Hazards Analysis

Variable (n = 108)	Hazards ratio for ≥ 24 mm Hg	*P* Value
Model 1: All the eyes treated with repeated TA injections (n = 108)		
Age (years)	0.99 (0.95 to 1.02)	.410
Hypertension	0.71 (0.33 to 1.29)	.276
IVI included	2.60 (1.30 to 4.83)	.010*
ΔIOP after 1st injection (mm Hg)	1.18 (1.04 to 1.30)	.011*
Model 2: Eyes with repeated STIs (n = 100)		
Age (years)	1.03 (0.98 to 1.08)	.247
Hypertension	0.82 (0.37 to 1.58)	.557
STI (mg)	1.07 (1.01 to 1.18)	.033*
ΔIOP after 1st injection (mm Hg)	1.45 (1.17 to 1.85)	.0006*

IVI = intravitreal injection of triamcinolone acetonide; STI = sub-Tenon capsule injection of triamcinolone acetonide; ΔIOP = maximal IOP minus baseline IOP.
Hazards ratio is shown with 95% confidence interval.
*$P < .05$.

Of the 108 eyes treated with a second injection, 16 (14.8%) had IOP elevation to 24 mm Hg or higher. Data before the second TA treatment for the group with elevation of less than 24 mm Hg and the group with T3 elevation of 24 mm Hg or higher are shown in Table 3. The 24 mm Hg or higher group included fewer patients with histories of hypertension, more eyes treated with the inclusion of IVI, and higher ΔIOP after the first injection. Cox proportional hazards regression analysis showed that the inclusion of IVI (HR, 2.60; 95% CI, 1.30 to 4.83; P = .010) and higher ΔIOP after the first injection (HR,1.18 per mm Hg; 95% CI, 1.04 to 1.30; P = .011) were risk factors for IOP elevation after the additional TA injection T4 (Table 4; Model 1). In eyes treated with two STI injections (n = 100), an increased dose administered by STI (HR, 1.07 per mg; 95% CI, 1.01 to 1.18; P = .033) and higher ΔIOP after the first injection (HR, 1.45 per mm Hg; 95% CI, 1.17 to 1.85; P = .0006) were shown to be risk factors (Table 4; Model 2).

DISCUSSION

THIS STUDY INVESTIGATED THE RISK FACTORS OF IOP ELEvation following topical TA injection. Cox proportional hazards regression analysis of 427 eyes showed that younger age (HR, 0.96 per year; 95% CI, 0.95 to 0.98), TA treatment including IVI (HR, 1.89; 95% CI, 1.41 to 2.52),

and higher baseline IOP (HR, 1.15 per year; 95% CI;1.05 to 1.27) were risk factors for elevated IOP of 24 mm Hg or higher. These risk factors were also observed in the 201 eyes treated with either 20 mg by STI or a combination of 20 mg by STI and 4 mg by IVI. TA dose dependency for the frequency of IOP elevation was identified by multivariate analyses for 333 eyes treated by STI (1.07 per mg; 95% CI, 1.03 to 1.12) and 57 eyes treated by IVI (1.64 per mg; 95% CI, 1.09 to 3.39). Moreover, multivariate analyses in eyes after two TA treatments showed that TA treatment including IVI, higher ΔIOP after the first TA injection, and a higher dose administered by STI were risk factors.

Several reports have discussed the rates of IOP elevation after TA injection, and have identified potential risk factors. Retrospective studies examining IVI-induced IOP elevation reported that treatment with 20 mg by IVI induced IOP of more than 21 mm Hg in 112 of 272 patients (41.2%),[1] and that 4 mg by IVI induced IOP elevation by 30% or more in 267 of 528 eyes (50.6%),[12] IOP elevation to 24 mm Hg or higher in 36 of 89 patients (40.4%),[6] and IOP elevation to more than 21 mm Hg, or by more than 5 mm Hg, in 26 of 60 patients (43.3%).[20] These results indicate that higher baseline IOP values[6,12] and younger age[1,20] are risk factors for IVI-induced IOP elevation.

By contrast, retrospective studies of STI-induced IOP elevation showed levels equal to or more than 6 mm Hg, or IOP levels of more than 20 mm Hg, in nine of 49 eyes (18.4%),[13] and IOP elevation of equal to or more than 5 mm Hg in 19 of 43 eyes (44.2%).[8] In our previous retrospective study, 40 mg by STI induced high IOP of 24 mm Hg or above in 26 of 115 eyes (22.6%).[15] Younger age[15] and a history of diabetes mellitus[13] are reported risk factors for STI-induced IOP elevation. However, to determine in detail the influence of risk factors, including the dose and route of TA administration, it will be necessary to carry out statistical analysis on a larger number of eyes treated with TA at multiple clinical centers. In this meta-study, to determine the TA-induced IOP elevation more exactly, we excluded eyes with other risk factors for IOP elevation, such as glaucoma, ocular hypertension, uveitis, steroid administration, and recent histories of intraocular surgery. Moreover, TA-induced IOP elevation obtained using noncontact pneumotonometry was confirmed using a Goldmann applanation tonometer. Taken together, our retrospective results reflect the detailed characterization of TA-induced IOP elevation.

No previous large-scale clinical studies have confirmed the risk factors for TA-induced IOP, or examined the effects of the amount of TA administered and the interaction between STI and IVI. The present study not only confirmed that younger age and higher baseline IOP risk factors,[1,6,12,20] but also revealed that IVI induces IOP elevation more frequently than STI, as well as demonstrating the dose dependency for TA-induced IOP elevation. However, no correlations with gender, medical history of hypertension, diabetes mellitus, cataract surgery, or vitrectomy were observed in the analyses for the risk factors. Although some reports have shown that diabetes mellitus is a risk factor for corticosteroid-induced IOP elevation,[13,21] others have shown that it is not significant. A previous randomized diabetes mellitus clinical trial conducted by Palmberg[22] showed that the history of diabetes mellitus was not associated with glaucoma. Our results seem to agree with this. In addition, it could be speculated that the lens and the vitreous affect the diffusion of TA in the ocular tissue; however, no reports (including our present results) suggest that the histories of cataract surgery and vitreous surgery influence TA-induced ocular hypertension.

Interestingly, IVI and ΔIOP are risk factors for IOP elevation in eyes treated with repeated TA injections. IOP elevation is also frequently associated with a higher dose of repeated STI treatment. There are some reports concerning IOP elevation after repeated TA injection.[6,7,12] A study that retrospectively investigated 43 eyes treated repeatedly with 20 to 25 mg by IVI showed that no eyes with 21 mm Hg or less after the first TA injection exhibited more than 21 mm Hg after the second TA injection.[24] By contrast, another study previously reported that 28 of 43 eyes (65.1%) treated with a second TA injection showed an IOP elevation of 30% or more, which was not observed at the first TA injection.[12] In our present study, 15 of 16 eyes with IOP elevation after the second TA injection did not exhibit IOP elevation after the first TA injection. Our present data appear to agree with the latter study, although it showed that the risk factors for IOP elevation after the second TA injection were higher baseline IOP and male gender.[12]

The study presented here has several limitations. First, it shows the risk factors for IOP elevation and not for TA-induced visual field loss attributable to severe TA-induced ocular hypertension. We could not retrospectively quantify visual field loss in eyes with TA-induced IOP elevation because of the association with retinal macular diseases. Second, we did not statistically analyze the duration of IOP elevation in this study. In total, 44 of 50 eyes with IOP elevation in this study showed reversible IOP elevation, whereas six eyes were associated with persistent ocular hypertension in spite of anti-glaucomatous medical treatments. They were treated with trabeculectomy (two eyes) and trabeculotomy (four eyes), which is a surgical procedure effective for corticosteroid-induced glaucoma.[15,23] The six eyes included three treated with 8 mg by IVI, one treated with 4 mg by IVI, one treated with 4 mg by IVI plus 20 mg by STI, and one treated with 40 mg by STI. Persistent IOP elevation might be associated with IVI or high-dose treatment by STI. Third, it remains to be determined whether glaucoma and ocular hypertension are risk factors, as we excluded patients suffering from these disorders from the present study. Such patients might be more susceptible to TA-induced

IOP elevation. Actually, few cases with past histories of glaucoma and ocular hypertension were treated with TA injection. In our clinical centers, TA injection might have been avoided in the patients associated with glaucoma or ocular hypertension. Fourth, we could not evaluate worldwide differences as we only analyzed data from Japanese patients.

In conclusion, our case-control study indicates that younger patients, those with a higher baseline IOP, and those receiving higher doses of TA or intravitreally administered TA are more susceptible to corticosteroid-induced IOP elevation. Greater IOP elevation after the first injection is associated with frequent IOP elevations after the second TA injection.

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REFERENCES

1. Jonas JB, Kreissig I, Degenring R. Intravitreal triamcinolone acetonide for treatment of intraocular proliferative, exudative, and neovascular diseases. Prog Retin Eye Res 2005;24:587–611.
2. Kawaji T, Hirata A, Awai N, et al. Trans-tenon retrobulbar triamcinolone injection for macular edema associated with branch retinal vein occlusion remaining after vitrectomy. Am J Ophthalmol 2005;140:540–542.
3. Antcliff RJ, Spalton DJ, Stanford MR, Graham EM, Ffytche TJ, Marshall J. Intravitreal triamcinolone for uveitic cystoid macular edema: an optical coherence tomography study. Ophthalmology 2001;108:765–772.
4. Danis RP, Ciulla TA, Pratt LM, Anliker W. Intravitreal triamcinolone acetonide in exudative age-related macular degeneration. Retina 2000;20:244–250.
5. Greenberg PB, Martidis A, Rogers AH, Duker JS, Reichel E. Intravitreal triamcinolone acetonide for macular edema due to central retinal vein occlusion. Br J Ophthalmol 2002;86:247–248.
6. Smithen LM, Ober MD, Maranan L, Spaide RF. Intravitreal triamcinolone acetonide and intraocular pressure. Am J Ophthalmol 2004;138:740–743.
7. Jonas JB, Kreissig I, Degenring R. Intraocular pressure after intravitreal injection of triamcinolone acetonide. Br J Ophthalmol 2003;87:24–27.
8. Jea SY, Byon IS, Oum BS. Triamcinolone-induced intraocular pressure elevation: intravitreal injection for macular edema and posterior subtenon injection for uveitis. Korean J Ophthalmol 2006;20:99–103.
9. Benz MS, Albini TA, Holz ER, et al. Short-term course of intraocular pressure after intravitreal injection of triamcinolone acetonide. Ophthalmology 2006;113:1174–1178.
10. Ozkiris A, Erkilic K. Complications of intravitreal injection of triamcinolone acetonide. Can J Ophthalmol 2005;40:63–68.
11. Yamashita T, Uemura A, Kita H, Sakamoto T. Intraocular pressure after intravitreal injection of triamcinolone acetonide following vitrectomy for macular edema. J Glaucoma 2007;16:220–224.
12. Rhee DJ, Peck RE, Belmont J, et al. Intraocular pressure alterations following intravitreal triamcinolone acetonide. Br J Ophthalmol 2006;90:999–1003.
13. Hirooka K, Shiraga F, Tanaka S, Baba T, Mandai H. Risk factors for elevated intraocular pressure after trans-tenon retrobulbar injections of triamcinolone. Jpn J Ophthalmol 2006;50:235–238.
14. Kaushik S, Gupta V, Gupta A, Dogra MR, Singh R. Intractable glaucoma following intravitreal triamcinolone in central retinal vein occlusion. Am J Ophthalmol 2004;137:758–760.
15. Iwao K, Inatani M, Kawaji T, Koga T, Mawatari Y, Tanihara H. Frequency and risk factors for intraocular pressure elevation after posterior sub-Tenon capsule triamcinolone acetonide injection. J Glaucoma 2007;16:251–256.
16. Bakri SJ, Beer PM. The effect of intravitreal triamcinolone acetonide on intraocular pressure. Ophthalmic Surg Lasers Imaging 2003;34:386–390.
17. Gillies MC, Simpson JM, Billson FA, et al. Safety of an intravitreal injection of triamcinolone: results from a randomized clinical trial. Arch Ophthalmol 2004;122:336–340.
18. Park CH, Jaffe GJ, Fekrat S. Intravitreal triamcinolone acetonide in eyes with cystoid macular edema associated with central retinal vein occlusion. Am J Ophthalmol 2003;136:419–425.
19. Massin P, Audren F, Haouchine B, et al. Intravitreal triamcinolone acetonide for diabetic diffuse macular edema: preliminary results of a prospective controlled trial. Ophthalmology 2004;111:218–224.
20. Park HY, Yi K, Kim HK. Intraocular pressure elevation after intravitreal triamcinolone acetonide injection. Korean J Ophthalmol 2005;19:122–127.
21. Becker B, Bresnick G, Chevrette L, Kolker AE, Oaks MC, Cibis A. Intraocular pressure and its response to topical corticosteroids in diabetes. Arch Ophthalmol 1966;76:477–483.
22. Palmberg P. Screening for diabetic retinopathy. Diabetes Care 2001;24:419–420.
23. Honjo M, Tanihara H, Inatani M, Honda Y. External trabeculotomy for the treatment of steroid-induced glaucoma. J Glaucoma 2000;9:483–485.
24. Jonas JB, Degenring R, Kreissig I, Akkoyun I. Safety of intravitreal high-dose reinjections of triamcinolone acetonide. Am J Ophthalmol 2004;138:1054–1055.

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Intraocular Pressure–Lowering Effects and Safety of Topical Administration of a Selective ROCK Inhibitor, SNJ-1656, in Healthy Volunteers

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Objective: To investigate the effects and safety of topical administration of an ophthalmic solution of a selective Rho-associated coiled coil-forming protein kinase (ROCK) inhibitor, SNJ-1656, 0.003% to 0.1%, in healthy male adult volunteers.

Design: Randomized, double-masked, group-comparison, phase 1 clinical study. In the initial single-dose trial, 45 healthy volunteers were randomly subdivided into 5 groups and treated with SNJ-1656 in concentrations of 0.003%, 0.01%, 0.03%, 0.05%, and 0.1% in stepwise fashion. In the repeated-instillation trial, 36 healthy volunteers were assigned to receive SNJ-1656 ophthalmic solution at the following concentrations and dosages: 0.05% once daily, 0.1% once daily, 0.05% twice daily, or 0.1% twice daily. In our studies, the administration of the solution and subsequent examinations (including intraocular pressure [IOP] measurements) were performed in a double-masked fashion.

Results: After single-dose instillation of placebo or SNJ-1656, 0.003%, 0.01%, 0.03%, 0.05%, and 0.1%, the changes in IOP from the baseline were −0.91, −1.18, −1.48, −2.20 ($P = .04$ vs placebo), −1.48, and −1.98 mm Hg, respectively, at 2 hours, and −0.63, −0.95, −1.79, −2.26 ($P = .01$ vs placebo), −1.95, and −3.00 ($P < .001$ vs placebo) mm Hg, respectively, at 4 hours. Significant IOP reductions after repeated instillation were also found. On slitlamp examination during the trial, there were no significant adverse findings except hyperemia of the bulbar and palpebral conjunctiva after instillation.

Conclusion: This clinical study demonstrated that SNJ-1656 ophthalmic solution is a safe topical agent effective in reducing IOP in human eyes.

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NUMEROUS DRUGS TO lower intraocular pressure (IOP) have been developed and used to treat glaucoma. Among them, prostaglandin analogues and adrenergic α_1-receptor antagonists have been shown to lower IOP by increasing uveoscleral (unconventional) outflow of aqueous humor,[1,2] whereas adrenergic β-receptor blockers, α_2-receptor agonists, and carbonic anhydrase inhibitors have been shown to reduce IOP by inhibiting aqueous humor production.[3-5] Pilocarpine and other miotic agents are believed to reduce IOP by increasing transcanalicular (conventional) aqueous outflow caused by contraction of the ciliary muscle (CM).[6] However, no IOP-lowering drugs directly modulating conventional outflow have been used clinically to treat glaucoma.

Rho guanosine triphosphatase, a member of the Rho subgroup of the Ras superfamily, participates in signaling pathways that lead to formation of actin stress fibers and focal adhesions.[7] Rho is also involved in diverse physiological functions associated with cytoskeletal rearrangement related to cell shape, cell motility, cytokinesis, and smooth muscle contraction.[8] Recently, several putative target molecules of Rho have been identified as Rho effectors, including Rho-associated coiled coil-forming protein kinase, termed p160ROCK, and its isoform, ROKa/Rho kinase/ROCK II.[9,10] ROCK has been shown to be expressed in ocular tissues, including the trabecular meshwork (TM) and CM.[11] In our previous study,[11] we demonstrated that instillation of Y-27632, a selective ROCK inhibitor, significantly reduced IOP, of which mechanism was attributed to improved outflow.[11-13] Inhibition of ROCK activity has been shown to induce alterations in TM cellular responses such as migration, adhesion, and changes in cell shape.[11] Another selective ROCK inhibitor, Y-39983, 4-[(1*R*)-1-aminoethyl]-*N*-(1*H*-pyrrolo[2,3-*b*]pyridin-4-yl) benzamide monohydrochloride, is 30-

fold more potent in inhibiting ROCK activity and has similar IOP-lowering effects at lower concentrations than Y-27632.[14]

The purpose of this clinical trial was to investigate the IOP-lowering effects and safety of SNJ-1656, an ophthalmic solution of Y-39983, in a single-dose trial and a prolonged repeated-instillation trial. We report herein the first results, to our knowledge, of a clinical trial of an ophthalmic solution consisting of a selective ROCK inhibitor in human eyes.

METHODS

We conducted this clinical trial as a randomized, double-masked, group-comparison, phase 1 clinical study in accordance with the ethical principles of the Declaration of Helsinki. Included in this study were healthy Japanese male volunteers, aged 20 to 35 years. Subjects with any history of ocular disease (including glaucoma), ocular surgery, or severe ocular trauma considered inappropriate for participation were excluded from the study. In addition, we excluded subjects with a history of liver, kidney, heart, digestive organ, or respiratory organ disorders; hematological diseases; or drug hypersensitivity. The subjects were considered eligible to participate if they had no abnormalities on ocular examination (including IOP) in either eye on screening by ophthalmologists. Subjects with a corrected visual acuity of less than 20/20, a cup-disc ratio of 0.6 or more in both eyes, or a difference in the cup-disc ratio of 0.2 or more between the eyes were excluded. Body weight was required to be within 80% to 120% of standard body weight value, calculated with the formula:

(Height in Centimeters − 100) × 0.9 kg.

During the trial, subjects were prohibited from continuing all medical treatment and from wearing contact lenses. Smoking and ingestion of caffeine, alcohol, and grapefruit were also prohibited during the trial.

First, the single-dose trial of SNJ-1656 and placebo ophthalmic solution (vehicle of SNJ-1656) was conducted in stepwise fashion from July 6 to September 17, 2005, at Osaka Clinical Pharmacological Institute, Osaka. The study was begun at step 1 (SNJ-1656, 0.003%, and placebo). After the safety of the ophthalmic solution was confirmed by physician interviews, physical examinations, ophthalmologic monitoring, and laboratory tests, step 2 (SNJ-1656, 0.01%, or placebo) was started, followed in turn by steps 3 (SNJ-1656, 0.03%, or placebo), 4 (SNJ-1656, 0.05%, or placebo), and 5 (SNJ-1656, 0.1%, or placebo). Nine subjects for each step (6 in the test drug group and 3 in the placebo group) were included. SNJ-1656 (or placebo) was topically administered in both eyes at 9 AM. Intraocular pressure was measured with noncontact tonometry before instillation and at 1 (10 AM), 2 (11 AM), 4 (1 PM), 8 (5 PM), 12 (9 PM), and 24 (9 AM the following day) hours after instillation.

To investigate the safety of prolonged repeated administration of SNJ-1656, a 7-day repeated-instillation trial was conducted from January 14 to April 1, 2006, at Osaka Clinical Trial Hospital, Osaka. The study was conducted in stepwise fashion from steps 1 (SNJ-1656, 0.05%, or placebo once daily), 2-1 (SNJ-1656, 0.1%, or placebo once daily), 2-2 (SNJ-1656, 0.05%, or placebo twice daily), and 3 (SNJ-1656, 0.1%, or placebo twice daily). However, steps 2-1 and 2-2 were concurrently conducted because the daily exposure of drug in the 0.05% twice daily group is the same as that in the 0.1% once daily group. Nine subjects for each step (6 in the test drug group and 3 in placebo group) were included. Twice-daily instillation was performed in both eyes of the subjects at 9 AM and 9:30 PM during the first 6 days and 9 AM on the seventh day. Once-daily instillation was performed in both eyes at 9 AM on all 7 days. The IOPs were measured with noncontact tonometry before instillation and at 1 (10 AM), 2 (11 AM), 4 (1 PM), 8 (5 PM), and 12 (9 PM) hours after instillation in the morning during the 7-day trial and remeasured on the eighth day at 24 hours (9 AM the following day) after the last instillation.

To evaluate the safety of SNJ-1656, ophthalmologic findings and physiological conditions were examined during the trials. The palpebral and bulbar conjunctiva, cornea, anterior chamber, iris, and lens were examined with slitlamp microscopy at 9 AM, 10 AM, 1 PM, 5 PM, and 9 PM daily during the trial. Also, the ocular findings were scored according to the following criteria: 0 indicates no significant changes; 0.5, slight changes regarded as physiological; 1, mild changes requiring no treatment; 2, moderate changes requiring any treatment; and 4, severe changes requiring hospitalization. Pupil diameter was measured at constant illumination at 9 AM, 10 AM, 11 AM, 5 PM, and 9 PM. General physiological factors, including blood pressure, pulse, and body temperature, were also monitored at 9 AM, 10 AM, 1 PM, 5 PM, and 9 PM. Electrocardiograms were obtained at 9 AM and 11 AM. Ocular examinations included determination of best-corrected visual acuity, retinal fundus examination, full-field flash electroretinography (LE-1000; Tomey, Nagoya, Japan), examination of the corneal and conjunctival surfaces with fluorescein and rose bengal dye, the Schirmer lacrimal test, corneal endothelial cell count with a specular microscope (Noncon Robo Pachy SP-9000, Konan Medical Inc, Tokyo), determination of corneal thickness using pachymetry (Noncon Robo Pachy SP-9000), and hematological and urine examinations, all performed at 9 AM. In the repeated-instillation trial, slitlamp examination, Schirmer lacrimal and rose bengal tests, the measurement of pupil diameter, and the monitoring of physiological factors were performed on the first, third, fifth, and seventh days of the trial. An electrocardiogram was obtained on the first, second, fourth, sixth, and seventh days. All examinations were reperformed on the last day of the trial and 1 week after the trial. Slitlamp photography was performed at baseline and whenever abnormal findings were obtained on slitlamp examination results. If volunteers experienced abnormal ocular symptoms, the volunteers indicated them on the patient data sheets. To minimize the adverse effects of SNJ-1656 in the subjects, the study was performed in ascending order from steps 1 to 5 in the single-instillation trial and steps 1, 2-1, 2-2, and 3 in the repeated-instillation trial.

In our studies, the ophthalmological solution was administered and subsequent examinations (including IOP measurements) were performed in a double-masked fashion. Unless otherwise indicated, data are expressed as mean±SD.

RESULTS

IOP-LOWERING EFFECT IN SINGLE-DOSE TRIAL

In the single-instillation trial of SNJ-1656, the mean IOP at baseline was 14.05±2.53 mm Hg for the placebo group and, for the SNJ-1656 groups, 14.08±1.44 mm Hg for 0.003%, 13.73±1.49 mm Hg for 0.01%, 13.73±2.18 mm Hg for 0.03%, 13.19±1.35 mm Hg for 0.05%, and 13.42±2.73 mm Hg for 0.1%, with no significant differences among the groups. The IOP levels in eyes administered SNJ-1656 first decreased and then returned to baseline levels by 24 hours after instillation (**Figure 1**A). The change in IOP from the baseline was −0.91, −1.18, −1.48, −2.20, −1.48, and −1.98 mm Hg at 2 hours and −0.63, −0.95, −1.79, −2.26, −1.95, and −3.00 mm Hg at

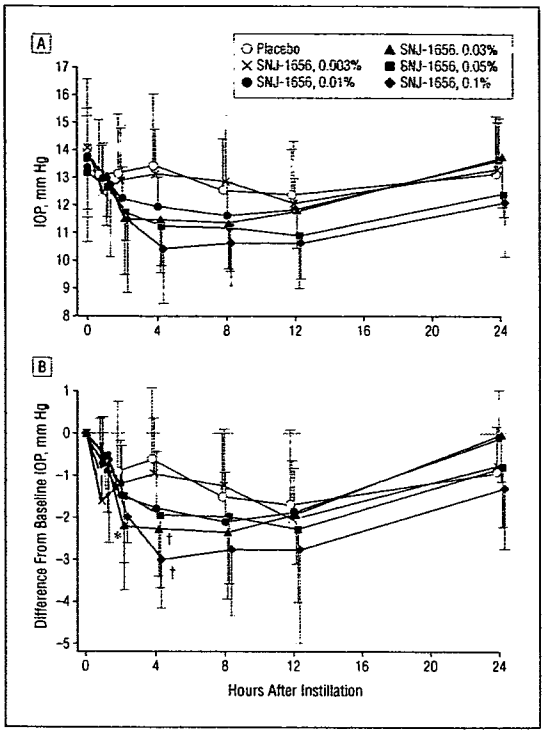

Figure 1. Levels of intraocular pressure (IOP) after single instillation of SNJ-1656. A, Levels of IOP decreased after instillation but were restored by 24 hours after instillation. B, Reduction of IOP after instillation of SNJ-1656 was dose dependent. Values are represented as mean±SD (SNJ-1656 group, 12 eyes in 6 subjects; placebo group, 30 eyes in 15 subjects). The significance of findings was evaluated by the Dunnett test (2 sided). *$P \leq .05$ compared with the placebo group. †$P \leq .01$ compared with the placebo group.

4 hours in the placebo, 0.003%, 0.01%, 0.03%, 0.05%, and 0.1% groups, respectively. Statistical analyses demonstrated significant differences in the magnitude of IOP reduction between the SNJ-1656– and placebo-treated eyes for 0.03% to 0.1% solutions ($P = .04$ at 2 hours and $P = .01$ at 4 hours for the 0.03% solution; $P < .001$ at 4 hours for the 0.1% solution [2-sided Dunnett test]) (Figure 1B). With SNJ-1656, 0.1%, mean IOP was 12.79±2.64, 11.44±2.58, 10.42±1.97, 10.63±1.56, 10.63±1.30, and 12.10±2.00 mm Hg at 1, 2, 4, 8, 12, and 24 hours after the instillation, respectively. Maximal IOP change with SNJ-1656, 0.1%, -3.00±1.16 mm Hg from the baseline IOP, was observed at 4 hours after instillation, and the IOP then slowly returned to near-baseline levels during the next 24 hours. The maximal IOP reduction after instillation of SNJ-1656, 0.1%, was larger than the reductions after instillation of lower concentrations (0.003% to 0.05%) of SNJ-1656. Similar, but weaker IOP-lowering effects were observed with lower concentrations of SNJ-1656.

SAFETY IN SINGLE-DOSE TRIAL

On slitlamp examination during the trial, there were no significant findings except hyperemia of the bulbar and palpebral conjunctiva in eyes treated with SNJ-1656 (**Figure 2**). This finding as a treatment-related adverse

Figure 2. Bulbar conjunctival hyperemia after instillation of SNJ-1656, 0.1%.

event was achieved in all 6 eyes with instillation of the 0.1% concentration and in 5 of the 6 eyes with instillation of the 0.05% concentration (**Table 1**). One subject with ocular hyperemia caused by SNJ-1656, 0.1%, experienced blurred vision, and another treated at this dose experienced photophobia. In contrast, with the 0.003% and 0.01% concentrations of SNJ-1656, fewer incidences of ocular hyperemia occurred, and no hyperemia occurred in the placebo group. The bulbar conjunctival hyperemia disappeared in all eyes, including those receiving the 0.1% concentration of SNJ-1656 (**Table 2**), by 12 hours after the instillation. Monitoring of pupil diameter showed no significant changes in pupil size during the trial. No significant changes were found between the preinstillation and postinstillation electroretinograms or the examination findings in the ocular fundus, the corneal endothelial cell count, or the corneal thickness. In addition, physiological examination results including blood pressure, pulse, body temperature, electrocardiograms (a wave, b wave, and amplitude), and hematological and urine testing showed no significant differences among volunteers administered SNJ-1656 or placebo.

IOP-LOWERING EFFECT OF REPEATED-INSTILLATION TRIAL

In the repeated-instillation trial, once-daily administration (steps 1 and 2-1) decreased IOP levels after instillation at 9 AM on each day in the SNJ-1656– and placebo-treated eyes (**Figure 3**A), whereas such a pattern of changes in IOP was unclear with twice-daily administration (steps 2-2 and 3; Figure 3B). The change in IOP from baseline was significantly larger in eyes treated with SNJ-1656 once daily (steps 1 and 2-1; Figure 3C) or twice daily (steps 2-2 and 3; Figure 3D) than in eyes treated with placebo. The mean changes in IOP from the baseline on the seventh day were -1.86±1.93, -2.78±0.98, and -3.70±1.12 ($P = .01$, vs placebo [2-sided Dunnett test]) mm Hg at 2 hours, and -1.58±1.56, -1.87±0.93, and -4.12±1.39 ($P < .001$) mm Hg at 4 hours in the groups receiving placebo and SNJ-1656 at concentrations of 0.05% (step 1) and 0.1% (step 2-1), once daily, respectively. Changes in IOP on the seventh day were -0.92±1.32, -3.45±1.18 ($P < .001$), and -2.51±1.74 ($P = .02$) mm Hg at 2 hours, and -1.19±1.10, -2.87±1.34

Table 1. Treatment-Related Adverse Events in Single-Dose Trial of SNJ-1656[a]

Symptom/Signs	SNJ-1656 Concentration				
	0.003% (n=6)	0.01% (n=6)	0.03% (n=6)	0.05% (n=6)	0.1% (n=6)
Bulbar conjunctival hyperemia	1	0	2	5	6
Palpebral conjunctival hyperemia	1	0	0	0	0
Blurred vision	0	0	0	0	1
Photophobia	0	0	0	0	1

[a]Data are expressed as number of volunteers with reported treatment-related adverse events. No treatment-related adverse events occurred in the placebo group (n=15).

Table 2. Change in Score of Bulbar Conjunctival Hyperemia After Instillation of SNJ-1656, 0.1%, in the Single-Dose Trial[a]

Time After Instillation, h	Score		
	0	0.5	1
0	12	0	0
1	0	0	12
4	0	4	8
8	2	7	3
12	4	8	0
24	8	4	0

[a]Data are expressed as number of eyes of the 12 eyes in 6 volunteers. No eyes achieved scores of 2 or 3. Scores are described in the "Methods" section.

($P=.01$), and -2.94 ± 1.75 ($P=.01$) mm Hg at 4 hours in the groups receiving placebo and SNJ-1656 at concentrations of 0.05% (step 2-2) and 0.1% (step 3), twice daily, respectively. The IOP-lowering effects of SNJ-1656 on the seventh day were similar to those on the first day in once- or twice-daily administration. The maximal changes in IOP in the SNJ-1656 groups were observed from 2 to 4 hours after instillation.

SAFETY IN REPEATED-INSTILLATION TRIAL

Hyperemia of the bulbar and palpebral conjunctiva as a treatment-related adverse event was observed in all steps in the repeated-instillation trial (**Table 3**). Some subjects treated with SNJ-1656, 0.1% (2 volunteers in step 2-1 and 1 volunteer in step 3), experienced blurred vision. One volunteer in step 3 complained of photophobia, ocular fatigue, and dryness of the eyes. In all of the subjects with these adverse effects, ocular hyperemia and other ocular symptoms disappeared spontaneously after the cessation of SNJ-1656 instillation. The bulbar conjunctival hyperemia disappeared in all eyes, including those receiving once-daily and twice-daily SNJ-1656, 0.1% (**Table 4**), by 8 hours after the instillation. There were no significant differences in pupil diameter between SNJ-1656 and placebo administrations. In addition, there were no other abnormal findings on slitlamp examination, no other ocular symptoms, and no significant abnormal physiological findings, including those for blood pressure, pulse, body temperature, and the electrocardiograms during the trial. There were no clinically significant changes from baseline in visual acuity, ocular fundus characteristics, corneal endothelial cell count, corneal thickness, electroretinographic findings, or laboratory values (hematologic analysis, blood chemistry, or urinalysis results) after the trial in the SNJ-1656 groups.

COMMENT

The IOP-lowering effects of SNJ-1656 in healthy adult volunteers were demonstrated in this study, which included a single-dose stage and a prolonged repeated-instillation stage. The study solution SNJ-1656 is an ophthalmic solution of Y-39983, a novel selective ROCK inhibitor, which has been reported to exhibit potent IOP-reducing activity in rabbits and monkeys.[14] Our findings obtained in this single-dose trial demonstrated that SNJ-1656 at concentrations ranging from 0.003% to 0.1% reduced IOP in a dose-dependent fashion without systemic or severe local ocular adverse effects. Mean IOPs in eyes treated with SNJ-1656, 0.03%, were significantly lower from 2 to 4 hours after instillation than were IOPs in eyes treated with placebo. The repeated-instillation trial also showed that IOP reductions from baseline were significantly larger in eyes with SNJ-1656 applications once daily and twice daily than in eyes treated with placebo. Maximal IOP reduction was observed from 2 to 4 hours after the instillation of SNJ-1656. No significant systemic adverse effects were observed. In addition, because IOP returned to baseline levels by 24 hours after instillation, and statistical difference from placebo in twice-daily administration was more than that in once-daily administration, twice-daily administration of this ophthalmic solution can be recommended as clinically useful.

In both the single- and repeated-instillation trials, the subjects experienced ocular treatment-related adverse events, although no systemic adverse events were observed. In our clinical trial, the most frequent adverse event was ocular hyperemia. Most of the subjects experienced no hyperemia or trace to mild hyperemia. In all cases, ocular hyperemia was transient and disappeared spontaneously after the cessation of SNJ-1656 instillation. Because the disappearance of hyperemia was confirmed in all eyes on slitlamp examination, SNJ-1656 did not seem to pose any safety problems for patients treated with lower concentrations. The occurrence of ocular hyperemia is

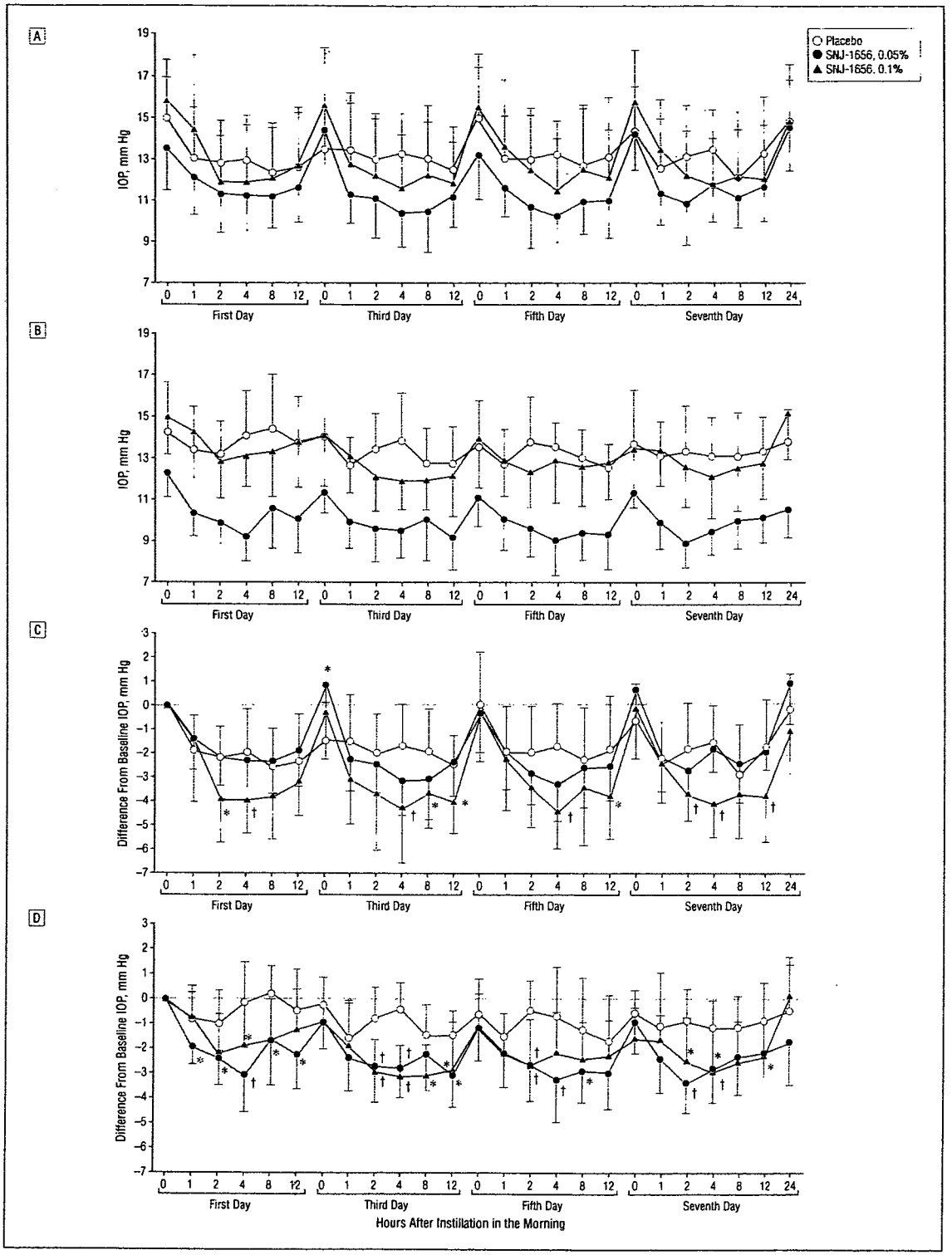

Figure 3. Levels of intraocular pressure (IOP) during repeated instillation of SNJ-1656. Levels of IOP in eyes with once-daily instillation of SNJ-1656 and placebo decreased after every 9 AM instillation (A), whereas diurnal changes in IOP were unclear with twice-daily administration (B). Reduction of IOP was significantly larger in eyes with SNJ-1656 administered once daily (C) or twice daily (D) than in eyes treated with placebo. Values are represented as mean±SD (12 eyes in 6 subjects). The significance of findings was evaluated by the Dunnett test (2 sided). *$P \leq .05$ compared with the placebo group. †$P \leq .01$ compared with the placebo group.

Table 3. Treatment-Related Adverse Events in Repeated-Instillation Trial of SNJ-1656[a]

	Once-Daily Administration (n=6)		Twice-Daily Administration (n=6)	
Symptom/Signs	SNJ-1656, 0.05%	SNJ-1656, 0.1%	SNJ-1656, 0.05%	SNJ-1656, 0.1%
Bulbar conjunctival hyperemia	2	6	5	5
Palpebral conjunctival hyperemia	1	3	3	1
Blurred vision	0	2	0	1
Photophobia	0	0	0	1
Ocular fatigue	0	0	0	1
Dryness of the eyes	0	0	0	1

[a] Data are expressed as number of volunteers with reported treatment-related adverse events. No treatment-related adverse events occurred in the placebo group.

Table 4. Change in Score of Bulbar Conjunctival Hyperemia After Instillation of SNJ-1656, 0.1%, on the Seventh Day in the Repeated-Instillation Trial[a]

	Score		
Time After Instillation, h	0	0.5	1
	Once-Daily Instillation		
0	12	0	0
1	0	4	8
4	3	8	1
8	9	3	0
12	12	0	0
24	12	0	0
	Twice-Daily Instillation		
0	12	0	0
1	2	4	6
4	6	6	0
8	10	2	0
12	12	0	0
24	12	0	0

[a] Data are expressed as number of eyes in the 12 eyes in 6 volunteers. No eyes achieved scores of 2 or 3. Scores are described in the "Methods" section.

consistent with findings in our previous animal experiments, in which similar conjunctival hyperemia (and minor hemorrhage) was found in rabbits and monkeys after frequent instillation of higher doses of SNJ-1656.[14] Hyperemia may be the result of relaxation of the blood vessels because ROCK inhibition induces smooth muscle relaxation.[11] Also, sporadic subconjunctival hemorrhage may be caused by impairment of barrier function or morphologic changes in vascular endothelial cells.[14] There were no clinically relevant effects of SNJ-1656 on visual acuity, ocular fundus characteristics, corneal endothelial cell count, corneal thickness, or electroretinographic findings. In addition, no clinically significant effects on blood pressure, pulse, body temperature, or electrocardiographic findings were noted with administration of SNJ-1656. The results of this study thus indicate that the IOP-lowering efficacy of SNJ-1656 was significant in healthy volunteers, and that adverse effects of its administration did not matter. Because we observed no systemic adverse effects in this study, we believe that the use of SNJ-1656 is safe, even for patients with systemic disease.

Aqueous outflow in the conventional pathway is regulated by the contraction and relaxation of the CM, and also by the TM, which possesses smooth musclelike properties.[15] It is thought that CM contraction distends the TM and increases aqueous outflow, whereas TM contraction decreases aqueous outflow.[6] Aqueous outflow is thus inversely influenced by the contractility of TM and CM. The contraction and relaxation of smooth muscle are regulated by myosin light chain phosphorylation/dephosphorylation. ROCK is involved in one of the major pathways of myosin light chain phosphorylation and is thought to regulate actomyosin-based contractility in many types of cells by phosphorylation of ROCK substrates.[16-18] Involvement of ROCK in control of IOP via regulation of the aqueous conventional outflow pathway has principally been demonstrated by 2 types of evidence: effects on the cellular behavior of TM, and the contribution of ROCK to the contractility of CM and TM. Recent studies have indicated that cytoskeletal drugs, including ROCK inhibitors, decrease aqueous outflow resistance by modulating cytoplasmic fibers.[19] In previous studies,[11,20] we found that the selective ROCK inhibitor Y-27632 causes alterations in cell shape; decreases actin stress fibers and focal adhesions in cultured human TM cells; elicits profound effects on TM cell activities, including adhesion, gel contraction, and cell motility; and decreases IOP in rabbit eyes. It has also been shown that Y-27632 increases aqueous outflow in enucleated, perfused porcine eyes[21] and that topical application of Y-39983 significantly decreases IOP in monkey eyes.[22] The inhibitors Y-27632 and Y-39983 induce relaxation of carbachol-contracted rabbit CM strips and TM[11,13] and contract monkey TM, exhibiting involvement of phosphorylation of myosin phosphatase by ROCK.[23] Collectively, these findings suggest that TM is a target for the development of new cytoskeletal drugs, including ROCK inhibitors, for new treatment of glaucoma. Based on the findings of the present study, SNJ-1656 can be considered a candidate drug for lowering IOP by increasing conventional outflow with few adverse effects.

In conclusion, our findings demonstrated that SNJ-1656 is a safe topical agent that is effective in reducing IOP in healthy adult volunteers. However, because our

trial was attempted primarily to evaluate the safety of SNJ-1656 in healthy subjects, further clinical trials will be required for elucidation of IOP-lowering effects in patients with ocular hypertension and/or primary open-angle glaucoma.

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REFERENCES

1. Gabelt BT, Kaufman PL. Prostaglandin $F_{2\alpha}$ increases uveoscleral outflow in the cynomolgus monkey. *Exp Eye Res.* 1989;49(3):389-402.
2. Oshika T, Araie M, Sugiyama T, Nakajima M, Azuma I. Effect of bunazosin hydrochloride on intraocular pressure and aqueous humor dynamics in normotensive human eyes. *Arch Ophthalmol.* 1991;109(11):1569-1574.
3. Coakes RL, Brubaker RF. The mechanism of timolol in lowering intraocular pressure in the normal eye. *Arch Ophthalmol.* 1978;96(11):2045-2048.
4. Toris CB, Gleason ML, Camras CB, Yablonski ME. Effects of brimonidine on aqueous humor dynamics in human eyes. *Arch Ophthalmol.* 1995;113(12):1514-1517.
5. Lippa EA, Carlson LE, Ehinger B, et al. Dose response and duration of action of dorzolamide, a topical carbonic anhydrase inhibitor. *Arch Ophthalmol.* 1992;110(4):495-499.
6. Gaasterland D, Kupfer C, Ross K. Studies of aqueous humor dynamics in man, IV: effects of pilocarpine upon measurements in young normal volunteers. *Invest Ophthalmol.* 1975;14(11):848-853.
7. Nobes C, Hall A. Regulation and function of the Rho subfamily of small GTPases. *Curr Opin Genet Dev.* 1994;4(1):77-81.
8. Narumiya S. The small GTPase Rho: cellular functions and signal transduction. *J Biochem (Tokyo).* 1996;120(2):215-228.
9. Ishizaki T, Naito M, Fujisawa K, et al. p160ROCK, a Rho-associated coiled-coil forming protein kinase, works downstream of Rho and induces focal adhesions. *FEBS Lett.* 1997;404(2-3):118-124.
10. Nakagawa O, Fujisawa K, Ishizaki T, Saito Y, Nakao K, Narumiya S. ROCK-I and ROCK-II, two isoforms of Rho-associated coiled-coil forming protein serine/threonine kinase in mice. *FEBS Lett.* 1996;392(2):189-193.
11. Honjo M, Tanihara H, Inatani M, et al. Effects of Rho-associated protein kinase inhibitor, Y-27632, on intraocular pressure and outflow facility. *Invest Ophthalmol Vis Sci.* 2001;42(1):137-144.
12. Rao PV, Deng PF, Kumar J, Epstein DL. Modulation of aqueous humor outflow facility by the Rho-kinase-specific inhibitor Y-27632. *Invest Ophthalmol Vis Sci.* 2001;42(5):1029-1037.
13. Waki M, Yoshida Y, Oka T, Azuma M. Reduction of intraocular pressure by topical administration of an inhibitor of the Rho-associated protein kinase. *Curr Eye Res.* 2001;22(6):470-474.
14. Tokushige H, Inatani M, Nemoto S, et al. Effects of topical administration of Y-39983, a selective Rho-associated protein kinase inhibitor, on ocular tissues in rabbits and monkeys. *Invest Ophthalmol Vis Sci.* 2007;48(7):3216-3222.
15. Wiederholt M, Thieme H, Stumpff F. The regulation of trabecular meshwork and ciliary muscle contractility. *Prog Retin Eye Res.* 2000;19(3):271-295.
16. Amano M, Ito M, Kimura K, et al. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem.* 1996;271(34):20246-20249.
17. Kimura K, Ito M, Amano M, et al. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science.* 1996;273(5272):245-248.
18. Kureishi Y, Kobayashi S, Amano M, et al. Rho-associated kinase directly induces smooth muscle contraction through myosin light chain phosphorylation. *J Biol Chem.* 1997;272(19):12257-12260.
19. Tian B, Geiger B, Epstein DL, Kaufman PL. Cytoskeletal involvement in the regulation of aqueous humor outflow. *Invest Ophthalmol Vis Sci.* 2000;41(3):619-623.
20. Koga T, Koga T, Awai M, Tsutsui J, Yue BY, Tanihara H. Rho-associated protein kinase inhibitor, Y-27632, induces alterations in adhesion, contraction and motility in cultured human trabecular meshwork cells. *Exp Eye Res.* 2006;82(3):362-370.
21. Rao PV, Deng P, Sasaki Y, Epstein DL. Regulation of myosin light chain phosphorylation in the trabecular meshwork: role in aqueous humour outflow facility. *Exp Eye Res.* 2005;80(2):197-206.
22. Tokushige H. ROCK inhibitor and glaucoma. *Bio Clinica.* 2002;17(13):1191-1194.
23. Fukiage C, Mizutani K, Kawamoto T, Azuma M, Shearer TR. Involvement of phosphorylation of myosin phosphatase by ROCK in trabecular meshwork and ciliary muscle contraction. *Biochem Biophys Res Commun.* 2001;288(2):296-300.

Tissue Type Plasminogen Activator Facilitates NMDA-Receptor–Mediated Retinal Apoptosis through an Independent Fibrinolytic Cascade

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Purpose. To investigate the association between apoptosis and the fibrinolytic system in retinal cell damage.

Methods. Tissue type plasminogen activator-deficient ($tPA^{-/-}$), urokinase type plasminogen activator-deficient ($uPA^{-/-}$), plasminogen activator inhibitor-1-deficient ($PAI\text{-}1^{-/-}$), α2 antiplasmin-deficient ($\alpha2\ AP^{-/-}$) mice, and their wild-type counterparts were used. Retinal cell damage was induced by intravitreal injection of the excitotoxin *N*-methyl-D-aspartate (NMDA). The TdT-dUTP terminal nick-end labeling (TUNEL) method was used to examine retinal cell damage.

Results. $tPA^{-/-}$ mice were resistant to retinal cell damage caused by administration of NMDA, and $PAI\text{-}1^{-/-}$ mice were more injured than their wild-type. No significant difference was observed between $uPA^{-/-}$ or $\alpha2\ AP^{-/-}$ and their wild-type mice.

Conclusions. The results strongly suggest that endogenous tPA, but not uPA acts as a facilitator in NMDA-induced retinal cell damage, and that its mechanism may not be associated with cleavage of plasminogen into plasmin in the fibrinolytic cascade. (*Invest Ophthalmol Vis Sci.* 2005;46:1504–1507) DOI:10.1167/iovs.04-0595

Tissue-type plasminogen activator (tPA) is used for the clinical treatment of thromboembolic stroke to activate the fibrinolytic system. The major source of tPA is endothelial cells; however, tPA has also been detected in neurons.[1] Therapeutic intervention with tPA in the nervous system may represent a two-edged sword, since it has been reported that tPA also promotes neurodegeneration after intracerebral injection of excitotoxins such as glutamate,[2] and neuronal damage after a cerebral infarction is thought to be mediated by excitotoxins.[3–6]

Retinal ganglion cell death is a common feature of many ophthalmic disorders, such as glaucoma and central artery or vein occlusion. Glaucoma in humans and monkeys is associated with a significant elevation in vitreal glutamate concentration.[7] The mechanism underlying retinal cell death in these diseases is not well understood. They are likely to involve, at least in part, ischemia-reperfusion injury, and the injury after ischemia may be due in part to the action of glutamate as an excitotoxin.[8]

Recently, we reported that tPA-deficient ($tPA^{-/-}$) mice are resistant to the retinal cell damage induced by excitotoxins, especially NMDA.[9] This result indicates that tPA facilitates NMDA-induced retinal cell damage, but the mechanism(s) by which tPA promotes retinal cell damage induced by NMDA, remains unclear.

The blood fibrinolytic system, which degrades intravascular fibrin, is activated by tPA or urokinase-type plasminogen activator (uPA), which convert plasminogen into plasmin.[10] These plasminogen activators are antagonized by an endogenous factor, plasminogen activator inhibitor-1 (PAI-1), and plasmin is inhibited by α2 antiplasmin (α2 AP).

In this study, to detect the association of excitotoxin-induced retinal cell death and the fibrinolytic system, $tPA^{-/-}$, uPA-deficient ($uPA^{-/-}$), PAI-1-deficient ($PAI\text{-}1^{-/-}$), and α2 AP-deficient ($\alpha2\ AP^{-/-}$) mice and their wild-type counterparts were used. According to a method we previously described,[9] insult to the retina was delivered by intravitreal injection of NMDA, and the degree of neuronal damage was estimated by the TdT-dUTP terminal nick-end labeling (TUNEL) method.

Materials and Methods

Experiment Animals

Male $tPA^{-/-}$, $uPA^{-/-}$, $PAI\text{-}1^{-/-}$, $\alpha2\ AP^{-/-}$, and wild-type mice weighing 25 to 30 g (on C57BL/6 and SV129 backgrounds) were used in the present study. Deficient mice were generated by homologous recombination in embryonic stem cells, as described previously.[11] All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intravitreal Injection

Mice were anesthetized with intraperitoneal injections of 50 mg/kg pentobarbital sodium (Nembutal; Dainippon Pharmaceutical Co., Ltd., Osaka, Japan). According to the method we reported,[9] all animals were intravitreally injected with a 30-gauge needle 0.5 mm behind the limbus in the temporal region of the globe, through the conjunctiva and sclera. In animals with dilated pupils, it was possible to view the needle entering the vitreous. Both eyes of each mouse ($n = 6$–9) were routinely injected with 3 μL of 10 mM NMDA (30 nanomoles). The mice that had postoperative complications such as retinal hemorrhage, vitreous hemorrhage, and retinal detachment were excluded from the analysis.

Histology and TdT-dUTP Nick-End Labeling

The mice were killed with an overdose of pentobarbital sodium 12 hours after intravitreal injection. The eyes were enucleated and post-fixed overnight in phosphate-buffered 10% formalin and then embed-

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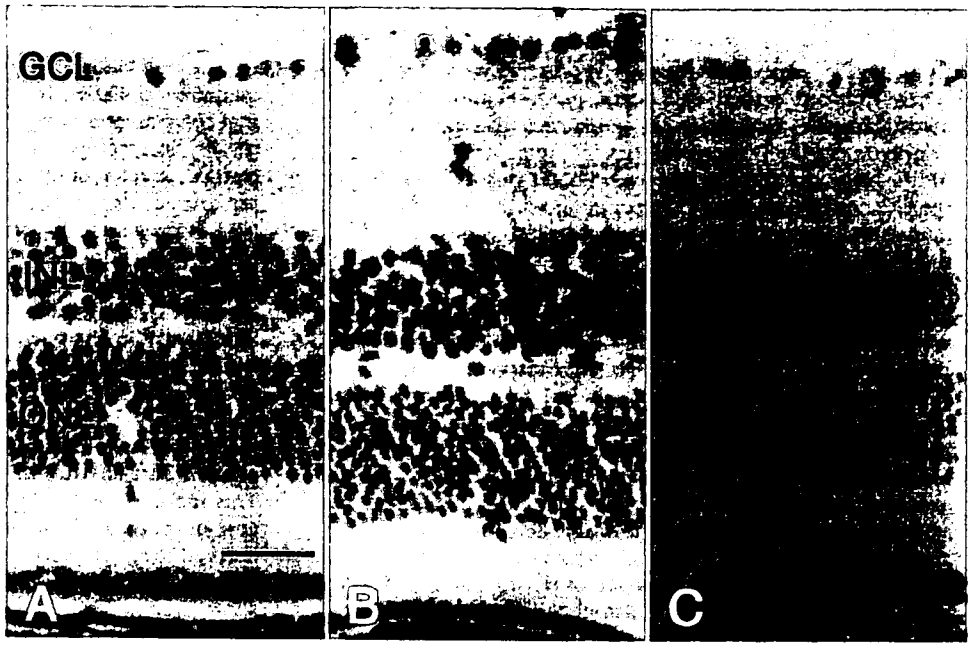

FIGURE 1. TUNEL staining of retinas at 12 hours after intravitreal administration of NMDA (30 nanomoles) or vehicle. (A) Vehicle in wild-type; (B) NMDA in wild-type; and (C) NMDA in tPA-deficient mice. TUNEL-positive cells appeared in the GCL and INL. Scale bar, 25 μm.

ded in paraffin. Sections 3 μm thick were cut along the vertical meridian through the optic nerve. To detect the retinal cells undergoing DNA fragmentation in the course of apoptosis, TUNEL staining was performed according to a method previously described.[12] The number of labeled cells in the ganglion cell layer (GCL), inner nuclear layer (INL), and outer nuclear layer (ONL) was counted in two central areas of the retina, approximately 250 μm long each, chosen from both sides of the optic nerve head.[13] Data were analyzed independently by two coauthors (MK, MN) in a blinded fashion. The number of TUNEL-positive cells per 250-μm length of the area in each retinal layer were averaged and plotted as the number of TUNEL-positive cells. The experimental results are expressed as the mean ± SD. Statistical analyses were performed by analysis of variance (ANOVA) with the Fisher protected least significant difference (Fisher's PLSD) test.

RESULTS

On the basis of preliminary experiments, we injected 30 nanomoles of NMDA intravitreally and dissected the eyes 12 hours later. To determine the association of apoptosis and the fibrinolytic system in retinal cell damage, we first compared NMDA-induced retinal damage in $tPA^{-/-}$ and wild-type mice. TUNEL-positive cells in both the GCL and INL in $tPA^{-/-}$ mice after intravitreal injection of NMDA were significantly fewer than in wild-type mice (Figs. 1, 2A). This result strongly indicates that endogenous tPA acts as a facilitator in NMDA-induced retinal cell damage.

To confirm the specificity of tPA, next we examined the contribution of another type of endogenous plasminogen activator, uPA. No significant difference in TUNEL-positive cells was observed between $uPA^{-/-}$ and wild-type mice in the GCL and INL after intravitreal injection of NMDA (Fig. 2B).

Endogenous tPA and uPA activity is negatively regulated by the endogenous inhibitory factor PAI-1. NMDA was injected intravitreally in $PAI\text{-}1^{-/-}$ mice to determine the role of endogenous PAI-1 in retinal damage. The number of TUNEL-positive cells in the GCL and INL after intravitreal injection of NMDA was significantly greater in $PAI^{-/-}$ mice than in wild-type mice (Fig. 2C).

tPA and uPA are serine proteases that convert plasminogen into plasmin, and α2 AP is an inhibitor of plasmin. To clarify whether plasmin is the key factor in the facilitative effect of tPA against the retinal cell damage induced by intravitreal injection of NMDA, we determined the contribution of endogenous α2 AP. After administration of NMDA, no significant difference was observed between $\alpha 2\ AP^{-/-}$ and wild-type mice (Fig. 2D).

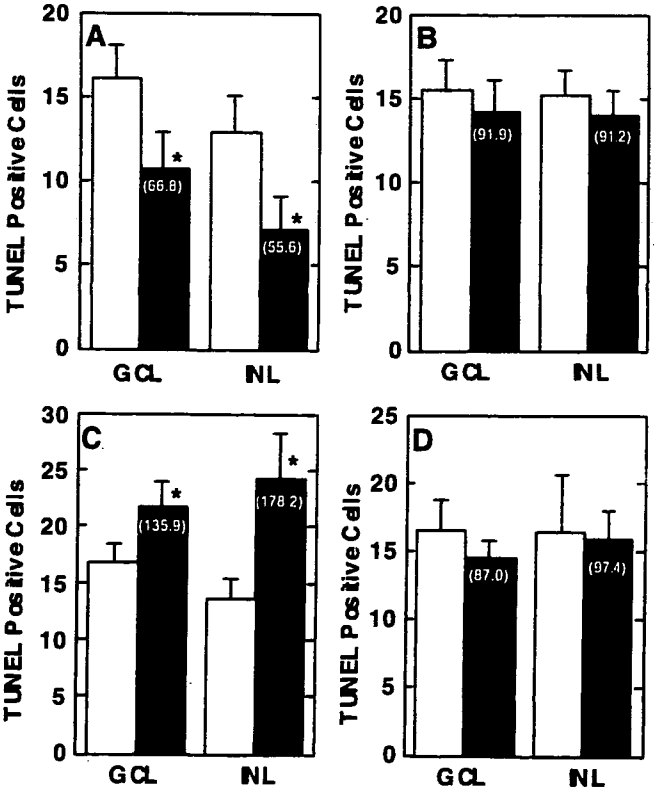

FIGURE 2. TUNEL-positive cells counted in the GCL and INL 12 hours after intravitreal injection of NMDA (30 nanomoles) in wild-type (□) and in (■) $tPA^{-/-}$ (A), $uPA^{-/-}$ (B), $PAI\text{-}1^{-/-}$ (C) or $\alpha 2\ AP^{-/-}$ (D) mice. Results are expressed as the mean ± SD (n = 12–18 eyes; six to nine mice). *Significant difference from the wild-type group at $P < 0.05$. Numbers in parenthesis indicate the percentage of TUNEL-positive cells versus each strain of wild-type mice.

Discussion

Using $tPA^{-/-}$ mice we recently reported that tPA facilitates NMDA-induced retinal cell death.[9] In the present study, to investigate the association of retinal cell damage and the fibrinolytic system, we used $tPA^{-/-}$, $uPA^{-/-}$, $PAI\text{-}1^{-/-}$, $\alpha 2AP^{-/-}$ mice, and their wild types. TUNEL-positive cells in both the GCL and INL in $tPA^{-/-}$ mice, but not in $uPA^{-/-}$ mice, after intravitreal injection of NMDA were significantly fewer than those in the wild type. Because endogenous tPA activity is negatively regulated by the endogenous inhibitory factor PAI-1, to determine the role of endogenous PAI-1 in retinal damage, we injected NMDA intravitreally into $PAI\text{-}1^{-/-}$ mice. TUNEL-positive cells in the GCL and INL after intravitreal injection of NMDA were significantly greater in $PAI^{-/-}$ mice than in wild-type mice. These results strongly suggest that tPA acts as a facilitator in NMDA-induced retinal cell damage, and that its mechanism may not be associated with cleavage of plasminogen into plasmin in the fibrinolytic cascade.

It has been reported that tPA and uPA are present in the retina. Tripathi et al.[14] examined various structures of human and monkey eyes for the presence of tPA by using the peroxidase-antiperoxidase immunohistochemical technique with a monoclonal antibody specific for human tPA. As a result, the anterior layers of the retina were weakly stained. In many of the tissues examined, uPA appeared to coexist with tPA. Tripathi et al.[15] investigated the presence of uPA in various structures of the human eye by using an immunohistochemical technique. A moderately intense to intermediate reaction product was seen in the anterior layers of the retina, a weak reaction product appeared in the posterior layers of the retina, and the retinal pigment epithelium contained both tPA and uPA. Therefore, the defect of PAI-1 would enhance endogenous tPA activity in the inner retina and lead to retinal cell death.

tPA is synthesized in basal conditions and is stored in vesicles.[16-19] However, in hippocampal CA1 neurons, tPA is undetectable in basal conditions, but is transiently induced after excitotoxic injury,[20] suggesting that induced tPA facilitates NMDA-induced CA1 damage. Although the precise role of constitutive or induced tPA in excitotoxic injury has not yet been determined, our results in tPA-deficient mice support the hypothesis that endogenous tPA is an essential factor in NMDA-mediated neuronal degeneration.

Our preliminary results showed that intravitreal injection of NMDA induces a dose-dependent loss of inner retinal elements, and there was a time-related appearance of TUNEL-positive nuclei in the inner retina. Lam et al.[21] showed intense labeling of nuclei between 12 and 24 hours after injection of NMDA. In the inner retina, retinal ganglion cells are particularly affected by extracellular glutamate, but a small percentage of cells in the INL are also stimulated. Although several different cell types in the INL express NMDA receptor subunits, only amacrine cells appear to express the same subunits as those detected in retinal ganglion cells. Amacrine cells may be adversely affected by NMDA.[22,23] The neuronal damage by NMDA is caused by calcium entry through the NMDA receptor, and elevation of intracellular calcium concentrations activate calcium-dependent protease, leading to neuronal death.[24,25]

tPA promotes NMDA-induced neuronal degeneration in brain hippocampal CA1 neurons.[26] Together with our present results, we can say that endogenous tPA is a common and important factor in NMDA-mediated neuronal degeneration. However, although it has been reported that tPA promotes not only NMDA-, but also transient ischemia-induced neuronal degeneration in the brain,[3] $tPA^{-/-}$ mice showed resistance to NMDA- but not transient ischemia-induced neuronal damage in the retina.[9] We therefore speculate that in addition to NMDA receptor activation, another mechanism is involved in transient ischemia-induced retinal damage.

The mechanism by which tPA modulates NMDA-receptor-mediated signaling is unknown, but Nicole et al.[27] reported that tPA potentiates signaling mediated by glutamatergic receptors by interacting with and cleaving the NR1 subunit of the NMDA receptor in the cerebral cortical neuron cultures. At the same time, they report that this interaction between tPA and NR1 is prevented by pretreatment with recombinant PAI-1, a protein that blocks the tPA catalytic site.[28] It has been suggested that tPA interacts with the NR1 subunit of the NMDA receptor through its catalytic site.[27] However, Matys and Strickland[29] questioned the data of Nicole et al.,[27] by suggesting that the anti-NR1 antibody used in their experiments was not specific for NR1 and may cross-react with plasminogen. They additionally indicated that Nicole et al.[27] used cultures maintained in serum-supplemented medium to coimmunoprecipitate and identify the NR1 subunit as a substrate for tPA. This method could have led to misidentification of plasminogen or plasmin bands as the NR1 subunit in its native or cleaved form. In response, Nicole et al. stated that the excitotoxic injury and cleavage experiments were all conducted in serum-free solutions. A casein gel zymography assay did not detect the presence of active plasmin, thereby excluding a possible contamination of their samples. Our results show that tPA increased NMDA-induced retinal cell damage, not associated with another function of tPA, cleavage of plasminogen into plasmin. Our data are consistent with the results of Nicole et al., but whether this effect is due to cleavage of the NR1 subunit by tPA is a subject of future studies.

In summary, tPA increased NMDA-induced retinal cell damage, and its mechanism is probably not associated with cleavage of plasminogen into plasmin in the fibrinolytic cascade. Retinal ganglion cell death is a common feature of many ophthalmic disorders, such as glaucoma and central artery or vein occlusion. Although the mechanism underlying retinal cell death in these diseases is not well understood, glaucoma in humans and monkeys is associated with a significant elevation in vitreal glutamate concentration.[7] Therefore, it is reasonable to hypothesize that retinal damage in ophthalmic diseases involves ischemia-reperfusion injury and the action of glutamate as an excitotoxin. Our study has provided key information on the mechanisms underlying retinal cell death and provides a basis for further investigation to identify fully all the mechanisms involved and novel therapeutic avenues for the treatment of various ophthalmic disorders.

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References

1. Sappino AP, Madani R, Huarte J, et al. Extracellular proteolysis in the adult murine brain. *J Clin Invest*. 1993;92:679-685.
2. Tsirka SE, Gualandris A, Amaral DG, et al. Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature*. 1995;377:340-344.
3. Wang YF, Tsirka SE, Strickland S, et al. Tissue plasminogen activator (tPA) increases neuronal damage after focal cerebral ischemia in wild-type and tPA-deficient mice. *Nat Med*. 1998;4:228-231.
4. Choi DW. Glutamate neurotoxicity and diseases of the nervous system. *Neuron*. 1988;1:623-634.
5. Meldrum B, Garthwaite J. Excitatory amino acid neurotoxicity and neurodegenerative disease. *Trends Pharmacol Sci*. 1990;11:379-387.
6. Tsirka SE, Rogove AD, Strickland S. Neuronal cell death and tPA. *Nature*. 1996;384:123-124.

7. Dreyer EB, Zurakowski D, Schumer RA, et al. Elevated glutamate in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol.* 1996;114:299-305.
8. Louzada-Junior P, Dias JJ, Santos WF, et al. Glutamate release in experimental ischaemia of the retina: an approach using microdialysis. *J Neurochem.* 1992;59:358-363.
9. Kumada M, Niwa M, Wang X, et al. Endogenous tissue type plasminogen activator facilitates NMDA-induced retinal damage. *Toxicol Appl Pharmacol.* 2004;200:48-53.
10. Collen D, Lijnen HR. Basic and clinical aspects of fibrinolysis and thrombolysis. *Blood.* 1991;78:3114-3124.
11. Carmeliet P, Schoonjans L, Kieckens L, et al. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature.* 1994;368:419-424.
12. Hara A, Niwa M, Iwai T, et al. Neuronal apoptosis studied by a sequential TUNEL technique: a method for tract-tracing. *Brain Res Brain Res Protoc.* 1999;4:140-146.
13. Daugeliene L, Niwa M, Hara A, et al. Transient ischemic injury in the rat retina caused by thrombotic occlusion-thrombolytic reperfusion. *Invest Ophthalmol Vis Sci.* 2000;41:2743-2747.
14. Tripathi BJ, Geanon JD, Tripathi RC. Distribution of tissue plasminogen activator in human and monkey eyes: an immunohistochemical study. *Ophthalmology.* 1987;94:1434-1438.
15. Tripathi RC, Tripathi BJ, Park JK. Localization of urokinase-type plasminogen activator in human eyes: an immunocytochemical study. *Exp Eye Res.* 1990;51:545-552.
16. Baranes D, López-García JC, et al. Reconstitution of the hippocampal mossy fiber and associational-commissural pathways in a novel dissociated cell culture system. *Proc Natl Acad Sci USA.* 1996;93:4706-4711.
17. Gualandris A, Jones TE, Strickland S, et al. Membrane depolarization induces calcium-dependent secretion of tissue plasminogen activator. *J Neurosci.* 1996;16:2220-2225.
18. Parmer RJ, Mahata M, Mahata S, et al. Tissue plasminogen activator (t-PA) is targeted to the regulated secretory pathway: catecholamine storage vesicles as a reservoir for the rapid release of t-PA. *J Biol Chem.* 1997;272:1976-1982.
19. Lochner JE, Kingma M, Kuhn S, et al. Real-time imaging of the axonal transport of granules containing a tissue plasminogen activator/green fluorescent protein hybrid. *Mol Biol Cell.* 1998;9:2463-2476.
20. Salles FJ, Strickland S. Localization and regulation of the tissue plasminogen activator-plasmin system in the hippocampus. *J Neurosci.* 2002;22:2125-2234.
21. Lam TT, Abler AS, Kwong JM, Tso MO. *N*-methy-D-aspartate (NMDA)-induced apoptosis in rat retina. *Invest Ophthalmol Vis Sci.* 1999;40:2391-2397.
22. Li Y, Schlamp CL, Nickells RW. Experimental induction of retinal ganglion cell death in adult mice. *Invest Ophthalmol Vis Sci.* 1999;40:1004-1008.
23. Siliprandi R, Canella R, Carmignoto G, et al. N-methyl-D-aspartate induced neurotoxicity in the adult rat retina. *Vis Neurosci.* 1992;8:567-573.
24. Hahn JS, Aizenman E, Lipton SA. Central mammalian neurons normally resistant to glutamate toxicity are made sensitive by elevated extracellular Ca2+: toxicity is blocked by the N-methyl-D-aspartate antagonist MK-801. *Proc Natl Acad Sci USA.* 1988;85:6556-6560.
25. Ferreira IL, Duarte CB, Carvalho AP. Ca2+ influx through glutamate receptor-associated channels in retina Ca2+ cells correlates with neuronal cell death. *Eur J Pharmacol.* 1996;302:153-162.
26. Tsirka SE, Rogove AD, Strickland S. Neuronal cell death and tPA. *Nature.* 1996;384:123-124.
27. Nicole O, Docagne F, Ali C, et al. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. *Nat Med.* 2001;7:59-64.
28. Gilis A, Declerck JP. Proteinase specificity and functional diversity in point mutants of plasminogen activator inhibitor 1. *J Biol Chem.* 1997;272:12662-12666.
29. Matys T, Strickland S. Tissue plasminogen activator and NMDA receptor cleavage. *Nat Med.* 2003;9:371-373.

The Tajimi Study Report 2

Prevalence of Primary Angle Closure and Secondary Glaucoma in a Japanese Population

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Purpose: To determine the prevalence of primary angle-closure glaucoma (PACG), secondary glaucoma (SG), and all glaucoma in a Japanese population as a part of the Tajimi Study.

Design: Population-based epidemiological survey.

Participants: A random sample of residents 40 years or older from Tajimi, Japan.

Intervention: Each subject underwent a screening program comprising an interview and an ophthalmic examination, including Goldmann applanation tonometry, slit-lamp examination, a van Herick test, fundus photography, and a screening visual field (VF) test using frequency-doubling technology. If glaucoma was suspected, the subject was referred for a definitive examination that included slit-lamp examination, gonioscopy, intraocular pressure measurement, a VF test, and optic disc and fundus examination. A diagnosis of PACG or SG was made based on slit-lamp examination, gonioscopy, optic disc appearance, and perimetric results.

Main Outcome Measures: Prevalences of PACG, SG, and all cases of glaucoma.

Results: Of 3870 eligible people, 3021 (78.1%) participated in the study. Estimated prevalences of PACG and SG in those over 40 years were 0.6% (95% confidence interval [CI], 0.4%–0.9%) and 0.5% (95% CI, 0.2%–0.7%), respectively. Prevalences of all glaucoma and glaucoma/suspected glaucoma were estimated to be 5.0% (95% CI, 4.2%–5.8%) and 7.5% (95% CI, 6.5%–8.4%), respectively.

Conclusions: Prevalences were 0.6%, 0.5%, and 5.0%, respectively, for PACG, SG, and all glaucoma in subjects over 40 years from Tajimi, Japan. *Ophthalmology 2005;112:1661–1669 © 2005 by the American Academy of Ophthalmology.*

Glaucoma is one of the most common causes of visual loss, and 22.5 million are estimated to suffer from glaucoma worldwide.[1,2] Primary angle-closure glaucoma (PACG) and primary angle closure (PAC) are more common in East Asian countries than in Western countries, and the former often results in bilateral blindness. Foster and Johnson[3] estimated that in China PACG accounted for 1.6 million cases of blindness, whereas primary open-angle glaucoma (POAG) accounted for 0.16 million cases. The prevalence of glaucoma depends on many factors, including ethnicity,

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age, gender, and geographic region. Further, differences in diagnostic instruments and methodologies for detecting the disease can markedly influence epidemiological findings. The prevalence of PACG was reported to be 1.4% in Mongolia,[4] 1.0% in Singapore,[5] and 0.5% to 1.08% in India,[6,7] whereas in Caucasians it ranged from 0.1% to 0.97%.[8–10] Primary angle-closure glaucoma develops in an age-dependent manner[11–15] and is more prevalent in women.[14–20] With the dark irides commonly seen in Asians, so-called creeping angle closure is thought to be the most common type of angle closure.[21–23] The majority of PAC is chronic and asymptomatic. For this reason, it is important to detect the disease early in its course.

Secondary glaucoma (SG) results from numerous ocular or systemic disorders or conditions, which may include uveitis, diabetic retinopathy, central retinal vein occlusion, and others. Clinically, SG often shows a poor response to ocular hypotensive agents or filtering surgery, especially in its late stages. Thus, like PACG, early detection is important in many types of SG to maximize the chance of a therapeutic response.

We recently reported that the prevalence of POAG, including normal-tension glaucoma, in the city of Tajimi, Japan was 3.9%,[24] which was about 50% higher than that previously estimated.[25] In this second report of the Tajimi Study, we focus on the age- and gender-specific prevalence of glaucomas other than POAG in the same population. In addition, we report the prevalence of all glaucoma.

Subjects and Methods

The subjects of the present study were identical to those in the first report of the Tajimi Study, which dealt with the prevalence of POAG in this population.[24] Thus, the fundamental methodology is identical to that reported previously, and is briefly summarized below.

Population Sampling

We screened the entire ≥40-year-old population of Tajimi City in central Japan. This epidemiological study was designed as a part of the Eye Health Care Project in Tajimi, and was conducted between September 2000 and October 2001. The investigation followed the tenets of the World Medical Association's Declaration of Helsinki and the municipal law of Tajimi City for protecting private information, and the study protocol was approved by the ethics committee of Tajimi City. Informed consent was obtained in written form from all the participants.

Of the 54 165 inhabitants 40 years or older in Tajimi City as of August 1, 2000, 4000 were selected randomly without stratification and were encouraged to participate in the epidemiological study. Although not stratified, the selected individuals were distributed evenly among all age groups.

Screening Examination

The screening examinations included not only ophthalmic examinations but also measurement of height, weight, and blood pressure. All examinations were performed by ophthalmologists trained in the diagnosis of glaucoma. Refractive status was measured using an autorefractometer (KP-8100PA, Topcon, Tokyo, Japan), and visual acuity (VA) was measured using a chart of Landolt rings at a distance of 5 m with refractive correction using the data obtained with an autorefractometer to start with. Central corneal thickness was measured using a specular-type pachymeter (SP-2000P, Topcon). Angle width was evaluated according to the van Herick method. Intraocular pressure (IOP) was measured 3 times by Goldmann applanation tonometry under topical anesthesia, and the median value was adopted. Digital color photographs of the fundus were taken with the pupil undilated using the IMAGEnet digital fundus camera system (NW6S, Topcon) with angles of 30° and 45°. The visual field (VF) was evaluated using a frequency-doubling technology (FDT) screener (Humphrey Instruments, San Leandro, CA) with the C-20-1 screening test. When participants were unable to come to the facilities, the doctors visited them at their homes and performed the examinations using a handheld slit lamp, direct and indirect ophthalmoscopes, and a Perkins applanation tonometer in the majority of the cases or a Tonopen XL (Bio-Rad Laboratories, Inc., Hercules, CA) in the remaining cases where Perkins tonometry could not be performed, and gonioscopy when necessary. As for the fundus examination, 3 examiners (the Photograph Screening Committee) independently evaluated the color fundus photographs for any abnormal findings including a glaucomatous optic disc appearance and nerve fiber layer defects. The rim border was determined based on shadows, gradations of color, texture, and the course of the blood vessels, and the vertical cup-to-disc (C/D) ratio and rim width were evaluated by units of 0.05 using a ruler. When at least 1 examiner noted any findings suggesting the presence of abnormality including glaucomatous changes, the subjects were recruited for the definitive examination.

Definitive Examination

Subjects were referred for definitive examination as having suspected glaucoma or other ocular diseases when their screening findings met one or more of the following criteria: corrected VA <

Table 1. Age-Specific Prevalence of Primary Angle-

Age Groups (yrs)	PACG (Percentage, 95% CI)		
	Male	*Female*	*All*
40–49	0/338 (0.0, 0.0–0.0)	0/445 (0.0, 0.0–0.0)	0/783 (0.0, 0.0–0.0)
50–59	0/427 (0.0, 0.0–0.0)	2/532 (0.4, 0.0–0.90)	2/959 (0.2, 0.0–0.50)
60–69	1/324 (0.3, 0.0–0.92)	5/360 (1.4, 0.18–2.60)	6/684 (0.9, 0.18–1.58)
70–79	3/190 (1.6, 0.0–3.35)	3/238 (1.3, 0.0–2.68)	6/428 (1.4, 0.29–2.51)
≥80	1/55 (1.8, 0.0–5.35)	4/112 (3.6, 0.13–7.01)	5/167 (3.0, 0.41–5.57)
All subjects	5/1334 (0.3, 0.01–0.67)	14/1687 (0.9, 0.45–1.31)	19/3021 (0.6, 0.35–0.91)

CI = confidence interval.

20/30; IOP > 19 mmHg (to refer all subjects whose IOP was statistically outside the normal limits); vertical C/D ratio of optic nerve head ≥ 0.6; difference of the vertical C/D ratio ≥ 0.2 between both eyes; rim width at superior portion (from the 11-o'clock position to the 1-o'clock position) or inferior portion (from the 5-o'clock position to the 7-o'clock position) ≤ 0.2 of the disc diameter; nerve fiber layer defect or splinter disc hemorrhage; any abnormal findings in the slit-lamp examination or fundus photographs, including low-quality fundus photographs; angle width ≤ grade 2 (van Herick method[26]); or at least one abnormal test point in the FDT VF test.

The definitive examination included slit-lamp examination, applanation tonometry, gonioscopy, and optic nerve head evaluation in a dark room with a Goldmann 2-mirror lens (Haag-Streit, Koeniz, Switzerland) and VF testing with the Humphrey Field Analyzer Central 30-2 Swedish Interactive Thresholding Algorithm Standard program (Humphrey Instruments). When the bottom of the angle was invisible, the part was observed with indentation to the opposite part of the angle using the 2-mirror lens. Unless the gonioscopy revealed a narrow angle of grade 2 or less by Shaffer's classification, the pupil was dilated to enable the taking of stereoscopic disc photographs (3-DX NM, Nidek, Gamagori, Japan) and the observation of the ocular fundus in detail. When the subject eye had a narrow angle, these examinations were carried out with the pupils undilated. Slit lamp examination, applanation tonometry, gonioscopy, and optic nerve head evaluation were carried out by glaucoma specialists who were voluntary council members of the Japan Glaucoma Society.

Evaluation of the Optic Disc, Nerve Fiber Layer Defect, and Visual Field

The details of the methods used here are described elsewhere[24] and are briefly summarized below.

Four glaucoma specialists (Photograph Reading Committee), to whom other information on the eyes was not revealed, evaluated the fundus photographs, including the stereoscopic photographs, of all the participants in a definitive examination. The vertical C/D ratio and rim width were again measured on the disc photographs by a glaucoma specialist (AI) with reference to the stereoscopic photographs. Each photograph was evaluated independently by 3 other examiners to confirm the measurements and to detect any nerve fiber layer defect, which was considered to be suggestive of glaucoma when its width at the disc edge was larger than a major retinal vessel, diverging in an arcuate or wedge shape. When the assessments by the examiners were not consistent, consensus was obtained by discussion while referring to the fundus color photographs and stereophotographs.

The results of the Humphrey Field Analyzer 30-2 Swedish Interactive Thresholding Algorithm Standard program were examined by 2 glaucoma specialists (the Visual Field Reading Committee), to whom other information on the eye in question was not revealed. Of all the VF data collected in the definitive examination (1799 eyes of 967 subjects), we excluded from evaluation only those apparently unreliable (fixation loss > 50%, false positive and false negative > 50%). Abnormal VF data were defined as the presence of at least one abnormal hemifield, which was determined based on the criteria proposed by Anderson and Patella.[27] The hemifield was judged to be abnormal when the pattern deviation probability plot showed a cluster of ≥3 non-edge contiguous points having sensitivity with a probability of <5% in the upper or lower hemifield and in one of these with a probability of <1%.

Diagnosis of Glaucomas

The final identification of glaucomas was based on clinical records obtained through all the examinations, by a panel of 6 glaucoma specialists. The presence of glaucoma was diagnosed only according to the results of evaluation of the optic disc and nerve fiber layer, and the VF as described elsewhere.[24] The diagnosis is briefly summarized below.

The criteria for glaucoma diagnosis were based upon the criteria of previous population studies.[28–31] First, the eye was diagnosed as having glaucoma (category 1) when the vertical C/D ratio of the optic nerve head was ≥0.7, the rim width at the superior portion (from the 11-o'clock position to the 1-o'clock position) or inferior portion (from the 5-o'clock position to the 7-o'clock position) was ≤0.1 of the disc diameter, the difference of the vertical C/D ratio was ≥0.2 between both eyes, or a nerve fiber layer defect was found, and the hemifield-based VF abnormality was compatible with the optic disc appearance or nerve fiber layer defect. Next, when the VF test result was not reliable or available, the diagnosis was obtained when the vertical C/D ratio was ≥0.9, the rim width at the superior portion (from the 11-o'clock position to the 1-o'clock position) or inferior portion (from the 5-o'clock position to the 7-o'clock position) was ≤0.05, or the difference of the vertical C/D ratio was ≥0.3 between both eyes (category 2). When a participant could not complete the VF testing and his or her optic disc was not visible, the diagnosis was made if the VA was ≤20/400 and the IOP percentile value for Japanese was 99.5—that is, ≥23 mmHg, or if the participant had a history of glaucoma surgery (category 3). The eye was diagnosed as having suspected glaucoma (glaucoma suspect) when the C/D ratios were ≥0.7 and <0.9, the rim width at the superior portion (from the 11-o'clock position to the 1-o'clock position) or inferior portion (from the 5-o'clock position to the 7-o'clock position) was ≤0.1 but >0.05 of the disc diameter, the difference of the vertical C/D ratio was ≥0.2 but <0.3 between both eyes, or a nerve fiber layer defect was found, and the VF test was not reliable or available or did not show a compatible hemifield-based defect. In a definitive

Closure Glaucoma (PACG) and Suspected PACG

Suspected PACG (Percentage, 95% CI)		
Male	*Female*	*All*
0/338 (0.0, 0.0–0.0)	0/445 (0.0, 0.0–0.0)	0/783 (0.0, 0.0–0.0)
1/427 (0.2, 0.0–0.68)	0/532 (0.0, 0.0–0.00)	1/959 (0.1, 0.0–0.30)
0/324 (0.0, 0.0–0.0)	1/360 (0.3, 0.0–0.83)	1/684 (0.2, 0.0–0.44)
1/190 (0.5, 0.0–1.56)	2/238 (0.8, 0.0–2.00)	3/428 (0.7, 0.0–1.49)
0/55 (0.0, 0.0–0.0)	1/112 (0.9, 0.0–2.63)	1/167 (0.6, 0.0–1.77)
2/1334 (0.1, 0.0–0.35)	4/1687 (0.3, 0.07–0.53)	6/3021 (0.2, 0.06–0.38)

Table 2. Age-Specific Prevalence of Primary Angle Closure (PAC) Excluding Primary Angle-Closure Glaucoma (PACG) and Suspected PACG

Age Groups (yrs)	PAC (Percentage, 95% CI)		
	Male	*Female*	*All*
40–49	0/338 (0.0, 0.0–0.0)	1/445 (0.2, 0.0–0.66)	1/783 (0.1, 0.0–0.38)
50–59	0/427 (0.0, 0.0–0.0)	2/532 (0.4, 0.0–0.90)	2/959 (0.2, 0.0–0.50)
60–69	1/324 (0.3, 0.0–0.92)	2/360 (0.6, 0.0–1.33)	3/684 (0.4, 0.0–0.94)
70–79	0/190 (0.0, 0.0–0.0)	6/238 (2.5, 0.53–4.51)	6/428 (1.4, 0.29–2.51)
≥80	0/55 (0.0, 0.0–0.0)	2/112 (1.8, 0.0–4.25)	2/167 (1.2, 0.0–2.85)
All subjects	1/1334 (0.1, 0.0–0.22)	13/1687 (0.9, 0.52–1.35)	14/3021 (0.5, 0.26–0.74)

CI = confidence interval.

diagnosis, anomalous discs, including tilted discs, were carefully excluded.

An occludable angle was defined as pigmented trabecular meshwork not visible in at least three quarters of the angle circumference. A diagnosis of PAC was made when the following criteria were met: at least one eye having a narrow angle of grade 2 or less by Shaffer's classification without other ocular findings that could have caused narrowing of the angle, and the existence of one or more of the following 4 conditions: IOP > 21 mmHg; a peripheral anterior synechia reaching the scleral spur or beyond; <90° of visibility of the pigmented trabecular meshwork in the primary position; and evidence of a history of an acute IOP rise, including the presence of iris atrophy, glaukomflecken, dilated nonreactive pupil, or a certified medical record of the subject having PAC. Primary angle-closure glaucoma or suspected PACG was diagnosed as PAC and glaucoma (category 1, 2, or 3) or suspected glaucoma as determined from the optic disc and VF findings as above.

Secondary glaucoma and suspected SG were diagnosed when the following criteria were met: positive history and/or ocular findings of intraocular inflammation, the presence of iris or angle neovascularization, presence of exfoliation materials on the iris margin or the lens surface, or other abnormal ocular findings that could cause prior or current IOP elevation and glaucoma (category 1, 2, or 3) or suspected glaucoma as determined above.

Early-onset developmental glaucoma and suspected cases were diagnosed when the following criteria were met: a developmental anomaly of the chamber angle that may cause IOP elevation; characteristic corneal changes such as diameter enlargement or Haab's striae; and/or related ocular anomalies such as seen in Axenfeld–Rieger syndrome, aniridia, and glaucoma (category 1, 2, or 3) or suspected glaucoma as determined above.

Primary angle closure, PACG and suspected PACG, SG and suspected SG, and early-onset developmental glaucoma and suspected cases were diagnosed on an individual basis. For example, if a patient had PACG in one eye and PAC in the other eye, the case was classified as PACG.

Data Analysis

All information was kept under the protection of participants' privacy at the Data Analysis Center of Tajimi Municipal Hospital. The data were double checked and validated through inspection and were analyzed using SAS version 6.12 (SAS Institute Japan, Tokyo, Japan) on a personal computer. Differences among the groups were evaluated using Student's *t* test. The Dunnett correction for multiple comparison was used when necessary. The prevalence of glaucoma and its confidence interval (CI) were calculated for each age group assuming that prevalences in participants and nonparticipants were equal. The prevalence rates were calculated by direct age-standardization from the population of Tajimi City. Association of age and gender with prevalence was evaluated using linear regression analysis and the chi-square test, respectively.

Results

Among the selected sample of 4000 subjects, 48 died and 82 were not actual residents or had moved from Tajimi City during the screening period. Of the 3870 remaining eligible persons, 3021 participated in the screening examinations, which resulted in a response rate of 78.1%. Response rates were similar in all age groups. There were 1065 subjects referred for a definitive examination after the initial screening examination. Of these 1065, 1051 received a definitive examination, whereas the remaining 14 declined or were unable to participate. For the 14 subjects who could not take the definitive examination, diagnosis was made based on the findings obtained in the screening examination, but none of them met the criteria of category 2 or glaucoma suspect. Of the 1065, 135 (4.5% of all subjects) showed grade 2 or less by the van

Table 3. Age-Specific Prevalence of Secondary Glaucoma (SG)

Age Groups (yrs)	SG (Percentage, 95% CI)		
	Male	*Female*	*All*
40–49	0/338 (0.0, 0.0–0.0)	1/445 (0.2, 0.0–0.66)	1/783 (0.1, 0.0–0.38)
50–59	0/427 (0.0, 0.0–0.0)	0/532 (0.0, 0.0–0.0)	0/959 (0.0, 0.0–0.0)
60–69	1/324 (0.3, 0.0–0.92)	1/360 (0.3, 0.0–0.83)	2/684 (0.3, 0.0–0.69)
70–79	2/190 (1.1, 0.0–2.50)	2/238 (0.8, 0.0–2.00)	4/428 (0.9, 0.02–1.84)
≥80	1/55 (1.8, 0.0–5.35)	1/112 (0.9, 0.0–2.63)	2/167 (1.2, 0.0–2.85)
All subjects	4/1334 (0.3, 0.0–0.57)	5/1687 (0.4, 0.09–0.61)	9/3021 (0.3, 0.13–0.51)

CI = confidence interval.

Herick method in at least one eye, of whom 19 (0.6%) showed grade 1 by the van Herick method. For females, numbers of subjects and age-specific prevalences of grade 2 or less by the van Herick method were 8 (1.8%), 27 (5.1%), 32 (8.9%), 28 (11.8%), 15 (13.4%), and 110 (6.5%) for those in their 40s, 50s, 60s, 70s, and 80s and above and for all cases, respectively; for males, these were 1 (0.3%), 8 (1.9%), 11 (3.4%), 4 (2.1%), 1 (1.8%), and 25 (1.9%), respectively. All cases with a van Herick grade of 1 had a gonioscopically narrow angle graded ≤2 by Shaffer's classification, and 81 cases (69.8%) with a van Herick grade of 2 also showed a narrow angle.

Tables 1 and 2 show age-specific prevalences of PACG and PAC. Overall prevalences of PACG and suspected PACG were 0.6% (95% CI, 0.4%–0.9%) and 0.2% (95% CI, 0.1%–0.4%), respectively. The prevalence significantly increased with age ($P<0.0001$ for PACG and $P<0.0001$ for PACG and suspected PACG, linear regression analysis) but did not significantly differ between women and men ($P = 0.1162$ for PACG and $P = 0.1023$ for PACG and suspected PACG, chi-square test). The prevalence of PAC including PACG and suspected PACG was 1.3% (95% CI, 0.9%–1.7%), and that of PAC excluding PACG and suspected PACG was 0.5% (95% CI, 0.3%–0.7%). The prevalence of PAC including PACG and suspected PACG significantly increased with age ($P<0.0001$, linear regression analysis). Furthermore, a significant difference was observed in the prevalence of PAC including PACG and suspected PACG between women and men ($P = 0.0028$, chi-square test). There were 19 cases of PACG, with 11 being diagnosed with category 1 criteria, 2 with category 2 criteria, and the remaining 6 with category 3 criteria. No cases showed gonioscopic findings suggestive of plateau iris syndrome. Central corneal thicknesses of both PACG and suspected PACG averaged 526±40 μm in the right eye and 530±39 μm in the left eye.

The prevalence of SG excluding exfoliation glaucoma was 0.3% (95% CI, 0.1%–0.5%; Table 3). The prevalence of suspected SG was 0.1% (95% CI, 0.0%–0.2%; Table 3). The prevalence significantly increased with age ($P = 0.0034$ for SG and $P = 0.0014$ for SG and suspected SG, linear regression analysis) but did not significantly differ between women and men ($P = 1.0$ for SG and $P = 0.8618$ for SG and suspected SG, chi-square test). Numbers of SG and suspected SG cases were 9 and 3, respectively. Of the 12 patients with SG or suspected SG, 8 had uveitis, 2 had ocular trauma, and 2 had iris and angle neovascularization. Of the 9 SG cases, 4 were diagnosed with category 1 criteria, 2 with category 2 criteria, and the remaining 3 with category 3 criteria. In addition to SG and suspected SG, 3 cases demonstrated secondary IOP elevation or a history of secondary IOP elevation with no sign of glaucoma.

Table 4 shows the prevalence of confirmed and suspected exfoliation glaucoma, defined as the presence of exfoliation materials on the iris margin or the lens surface and IOP elevation, and glaucoma (category 1, 2, or 3) or suspected glaucoma. Prevalences of exfoliation glaucoma and suspected cases were 0.2% (95% CI, 0.0%–0.3%) and 0.1% (95% CI, 0.0%–0.2%), respectively. The prevalence significantly increased with age ($P = 0.0183$ for exfoliation glaucoma and $P = 0.0073$ for exfoliation glaucoma and suspected cases, linear regression analysis) but did not significantly differ between women and men ($P = 0.2442$ for exfoliation glaucoma and $P = 0.4904$ for exfoliation glaucoma and suspected cases, chi-square test). The numbers of exfoliation glaucoma cases and suspected cases were 5 and 3, respectively. Of the 5 exfoliation glaucoma cases, 3 were diagnosed with category 1 criteria and 2 with category 2 criteria; none were diagnosed with category 3 criteria. No subjects showed secondary IOP elevation without a sign of glaucoma due to exfoliation syndrome. Table 5 demonstrates the prevalence of exfoliation syndrome excluding confirmed and suspected exfoliation glaucoma. The prevalence was 0.8% (95% CI, 0.5%–1.1%). The prevalence of exfoliation syndrome including confirmed and suspected exfoliation glaucoma significantly increased with age ($P<0.0001$, linear regression analysis) but did not differ between genders ($P = 0.7809$, chi-square test).

No patient was found to have confirmed or suspected early-onset developmental glaucoma.

As for the impact of glaucoma on the quality of vision, one PACG patient had VA in one eye of <20/400 (i.e., the World Health Organization's criterion for blindness), and there was one case each of uveitic, traumatic, and neovascular glaucoma with VA of <20/400 in one eye.

Based on the present and previous data,[24] the prevalence of all glaucoma (categories 1, 2, and 3) in Tajimi was calculated to be 5.0% (95% CI, 4.2%–5.8%), and that including suspected cases was 7.5% (95% CI, 6.5%–8.4%). Table 6 shows the standardized prevalence of glaucoma and suspected cases.

Discussion

Epidemiological studies should be performed based on clear definitions of the targeted diseases. Until recently, the nomenclature for PACG and related conditions such as PAC was not well established. In actuality, there was little information about glaucomatous optic nerve damage in many of the classic articles dealing with PACG. For that reason, care must be taken in the interpretation and comparison of the previous literature on PACG. Recently, a new epidemiologic definition clarifying angle closure has been proposed,[31,32] in which the term *glaucoma* is applied only to cases of glaucomatous optic neuropathy and its corresponding VF loss. These efforts toward a standardized definition of PAC will facilitate both the diagnosis and com-

and Suspected SG, Excluding Exfoliation Glaucoma

Suspected SG (Percentage, 95% CI)		
Male	*Female*	*All*
0/338 (0.0, 0.0–0.0)	0/445 (0.0, 0.0–0.0)	0/783 (0.0, 0.0–0.0)
0/427 (0.0, 0.0–0.0)	0/532 (0.0, 0.0–0.0)	0/959 (0.0, 0.0–0.0)
1/324 (0.3, 0.0–0.92)	1/360 (0.3, 0.0–0.83)	2/684 (0.3, 0.0–0.69)
0/190 (0.0, 0.0–0.0)	1/238 (0.4, 0.0–1.24)	1/428 (0.2, 0.0–0.68)
0/55 (0.0, 0.0–0.0)	0/112 (0.0, 0.0–0.0)	0/167 (0.0, 0.0–0.0)
1/1334 (0.1, 0.0–0.22)	2/1687 (0.1, 0.0–0.31)	3/3021 (0.1, 0.0–0.21)