decreased VEGF production. In addition, VEGF isoforms other than $VEGF_{121}$ and $VEGF_{165}$ might play a key role in mCNV. The antibody we used can detect free $VEGF_{121}$ and free $VEGF_{165}$. Therefore, we cannot deny the possibility that bound VEGF or other VEGF isoforms might play a key role in mCNV.

In the current study, we found a significantly lower mean VEGF concentration in the aqueous humour in patients with mCNV. To determine the pathogenesis of VEGF in mCNV, further studies are warranted of the local presence and intraretinal expression of VEGF in eyes with mCNV and a comparison of VEGF concentrations in the aqueous humour in patients with high myopia without mCNV.

Acknowledgements

This study was supported in part by a grant from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#21592255) and a grant from the Ministry of Health, Labour and Welfare.

References

Aiello LP, Avery RL, Arrigg PG et al. (1994): Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med **331**: 1480–1487.

Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA & Giust MJ (2006): Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology **113**: 363–372.

Avia MP, Weiter JJ, Jalkh AE, Trempe CL, Pruett RC & Schepens CL (1984): Natural history of choroidal neovascularization in degenerative myopia. Ophthalmology **91**: 1573–1581.

Blinder KJ, Blumenkranz MS, Bressler NM et al. (2003): Verteporfin therapy of subfoveal choroidal neovascularization in pathologic myopia: 2-year results of a randomized clinical trial – VIP report no 3. Ophthalmology **110**: 667–673.

Chan WM, Lai TY, Chan KP, Li H, Liu DT, Lam DS & Pang CP (2008): Changes in aqueous vascular endothelial growth factor and pigment epithelial-derived factor levels following intravitreal bevacizumab injections for choroidal neovascularization secondary to age-related macular degeneration or pathologic myopia. Retina **28**: 1308–1313.

Chan WM, Lai TY, Liu DT & Lam DS (2009): Intravitreal bevacizumab (Avastin) for myopic choroidal neovascularization: 1-year results of a prospective pilot study. Br J Ophthalmol **93**: 150–154.

Degenring RF & Jonas JB (2005): Photodynamic therapy in combination with intravitreal triamcinolone for myopic choroidal neovascularization. Acta Ophthalmol Scand **83**: 621.

Ergun E, Heinzl H & Stur M (2004): Prognostic factors influencing visual outcome of photodynamic therapy for subfoveal choroidal neovascularization in pathologic myopia. Am J Ophthalmol **138**: 434–438.

Ferrara N (2004): Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev **25**: 581–611.

Gharbiya M, Allievi F, Mazzeo L & Gabrieli CB (2009): Intravitreal bevacizumab treatment for choroidal neovascularization in pathologic myopia: 12-month results. Am J Ophthalmol **147**: 84–93.

Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M & Guyer DR (2004): VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med **351**: 2805–2816.

Hera R, Keramidas M, Peoc'h M, Mouillon M, Romanet JP & Feige JJ (2005): Expression of VEGF and angiopoietins in subfoveal membranes from patients with age-related macular degeneration. Am J Ophthalmol **139**: 589–596.

Hyodo I, Doi T, Endo H, Hosokawa Y, Nishikawa Y, Tanimizu M, Jinno K & Kotani Y (1998): Clinical significance plasma vascular endothelial growth factor in gastrointestinal cancer. Eur J Cancer **34**: 2041–2045.

Ikuno Y, Sayanagi K, Soga K, Sawda M, Tsujikawa M, Gomi F & Tano Y (2009): Intravitreal bevacizumab for choroidal neovascularization attributable to pathological myopia: one-year results. Am J Ophthalmol **147**: 94–100.

Ishibashi T, Hata Y, Yoshikawa H, Nakagawa K, Sueishi K & Inomata H (1997): Expression of vascular endothelial growth factor in experimental choroidal neovascularization. Graefes Arch Clin Exp Ophthalmol **235**: 159–167.

Jonas JB & Neumaier M (2007): Vascular endothelial growth factor and basic fibroblast growth factor in exudative age-related macular degeneration and diffuse diabetic macular edema. Ophthalmic Res **39**: 139–142.

Konstantinidis L, Mantel I, Pournaras JA, Zoqrafos L & Ambresin A (2009): Intravitreal ranibizumab (Lucentis) for the treatment of myopic choroidal neovascularization. Graefes Arch Clin Exp Ophthalmol **247**: 311–318.

Kvanta A, Algvere PV, Berglin L & Seregard S (1996): Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci **37**: 1929–1934.

Kwak N, Okamoto N, Wood JM & Campochiaro PA (2000): VEGF is major stimulator in model of choroidal neovascularization. Invest Ophthalmol Vis Sci **41**: 3158–3164.

Lam DS, Chan WM, Liu DT, Fan DS, Lai WW & Chong KK (2004): Photodynamic therapy with verteporfin for subfoveal choroidal neovascularisation of pathologic myopia in Chinese eyes: a prospective series of 1 and 2 year follow up. Br J Ophthalmol **88**: 1315–1319.

Lam DS, Leung KS, Mohamed S et al. (2007): Regional variations in the relationship between macular thickness measurements and myopia. Invest Ophthalmol Vis Sci **48**: 376–382.

Lopez PF, Sippy BD, Lamber HM, Thach AB & Hinton DR (1996): Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci **37**: 855–868.

Noma H, Funatsu H, Yamasaki M et al. (2005): Pathogenesis of macular edema with branch retinal vein occlusion and intraocular levels of vascular endothelial growth factor and interleukin-6. Am J Ophthalmol **140**: 256–261.

Rosenfeld PJ, Heier JS, Hantsbager G & Shams N (2006): Tolerability and efficacy of multiple escalating doses of ranibizumab (Lucentis) for neovascular age-related macular degeneration. Ophthalmology **113**: 623.

Salzmann M (1912): Anatomy and histology of the human eye ball in the normal state, its development and senescence (translated by E.V.L. Brown). Chicago: University of Chicago Press.

Sawada O, Kawamura H, Kakinoki M, Sawada T & Ohji M (2007): Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. Arch Ophthalmol **125**: 1363–1366.

Tong JP, Chan WM, Liu DT, Lai TY, Choy KW, Pang CP & Lam DS (2006): Aqueous humor levels of vascular endothelial growth factor and pigment epithelium-derived factor in polypoidal choroidal vasculopathy and choroidal neovascularization. Am J Ophthalmol **141**: 456–462.

Yoshida T, Ohno-Matsui K, Yasuzumi K, Kojima A, Shimada N, Futagami S, Tokoro T & Mochizuki M (2003): Myopic choroidal neovascularization: a 10 years follow-up. Ophthalmology **110**: 1297–1305.

Received on February 24th, 2009.
Accepted on July 10th, 2009.

Correspondence:
Osamu Sawada
Department of Ophthalmology
Shiga University of Medical Science
Seta Tukinowacho
Otsu
Shiga 520-2192
Japan
Tel: + 81 775 482 276
Fax: + 81 775 482 279
Email: osawada@belle.shiga-med.ac.jp

AQUEOUS VASCULAR ENDOTHELIAL GROWTH FACTOR AFTER INTRAVITREAL INJECTION OF PEGAPTANIB OR RANIBIZUMAB IN PATIENTS WITH AGE-RELATED MACULAR DEGENERATION

OSAMU SAWADA, MD, TAICHIRO MIYAKE, MD,
MASASHI KAKINOKI, MD, TOMOKO SAWADA, MD,
HAJIME KAWAMURA, MD, MASAHITO OHJI, MD

Purpose: The purpose of this study was to evaluate vascular endothelial growth factor (VEGF) concentrations in the aqueous humor of eyes after intravitreal injections of pegaptanib or ranibizumab in patients with age-related macular degeneration.

Methods: Aqueous humor samples were obtained from 16 eyes with choroidal neovascularization secondary to age-related macular degeneration before and after intravitreal injections of pegaptanib (0.3 mg; 5 eyes) and ranibizumab (0.5 mg; 11 eyes). The VEGF concentration was measured using an enzyme-linked immunosorbent assay using a primary antibody against $VEGF_{121}$ and $VEGF_{165}$.

Results: The VEGF concentrations in the aqueous humor of eyes with age-related macular degeneration ranged from 35.3 pg/mL to 142.4 pg/mL (mean ± standard deviation, 90.9 pg/mL ± 40.0 pg/mL) before the injection of pegaptanib and increased significantly, ranging from 298.2 pg/mL to 571.3 pg/mL (mean ± standard deviation, 452.0 pg/mL ± 106.4 pg/mL) 6 weeks after the injection ($P = 0.005$). The VEGF concentrations ranged from 47.2 pg/mL to 307.4 pg/mL (mean ± standard deviation, 125.9 pg/mL ± 77.2 pg/mL) before injection of ranibizumab and decreased to <31 pg/mL, the lower limit of detection, 4 weeks after injection.

Conclusion: The VEGF concentrations in the aqueous humor of eyes with age-related macular degeneration decreased after injections of ranibizumab and increased after injections of pegaptanib.

RETINA 30:1034–1038, 2010

Vascular endothelial growth factor (VEGF) is a pathogenic factor for formation of choroidal neovascularization (CNV) in neovascular age-related macular degeneration (AMD).[1–7] Intravitreal injection of anti-VEGF drugs is effective for treating neovascular AMD.[8–12] The anti-VEGF drugs used to treat neovascular AMD include bevacizumab (Avastin, Genentech, South San Francisco, CA), pegaptanib (Macugen, OSI/Eyetech and Pfizer, New York, NY), and ranibizumab (Lucentis, Novartis, Basel, Switzerland). Bevacizumab, a recombinant humanized monoclonal antibody against all VEGF isoforms and approved to treat colorectal cancer,[13] has been used for AMD as an off-label drug.[11,12] Pegaptanib, a 28-ribonucleotide aptamer conjugated to a polyethylene glycol moiety and a selective inhibitor of $VEGF_{165}$, has been approved as an intravitreal treatment for neovascular AMD.[2] Ranibizumab, a humanized antigen-binding portion of a murine anti-VEGF monoclonal antibody

From the Department of Ophthalmology, Shiga University of Medical Science, Shiga, Japan.

Supported, in part, by the Ministry of Education, Culture, Sports, Science and Technology of Japan grant (21592255) and a grant from the Ministry of Health, Labor, and Welfare.

The authors have no proprietary interest in any aspect of this study.

Reprint requests: Osamu Sawada, MD, Department of Ophthalmology, Shiga University of Medical Science, Seta Tukinowacho, Otsu, Shiga, 520–2192, Japan; e-mail: osawada@belle.shiga-med.ac.jp

with a mature high affinity for all VEGF isoforms, has been approved as an intravitreal treatment for neovascular AMD and is the first such treatment to improve visual acuity in neovascular AMD.[3,4] The efficacy of these drugs for neovascular AMD differs in effecting changes in the visual acuity, and the difference may depend on the VEGF concentration after treatment. We measured the aqueous VEGF concentration before and after intravitreal injections of pegaptanib and ranibizumab to elucidate this possibility.

Methods

In this prospective study, we measured the VEGF concentrations in the aqueous humor of 16 eyes of 16 patients (2 women and 14 men) with CNV secondary to AMD before and after intravitreal injections of anti-VEGF agents (5 eyes were treated with pegaptanib and 11 eyes were treated with ranibizumab). The mean patient age was 76.6 years (range, 61–95 years). The presence of CNV was confirmed with fluorescein angiography and indocyanine green angiography in all patients. No patient had been treated previously for AMD. Five eyes received intravitreal injections of pegaptanib (0.3 mg) 3 times every 6 weeks; 11 eyes received intravitreal injections of ranibizumab (0.5 mg) 3 times monthly.

Undiluted aqueous humor samples were obtained from the eyes of patients with AMD just before an intravitreal injection of ranibizumab and 6 weeks after the first injection of pegaptanib or 1 month after the first injection of ranibizumab. All sample collections were performed using a standard sterilization procedure that included topical povidone–iodine and levo-floxacin drops. The samples were stored in a freezer at −80°C until analysis.

The best-corrected visual acuity was measured and optical coherence tomography was performed before the first intravitreal injections and just before the second intravitreal injections of ranibizumab. The central foveal thickness (CFT) was measured by spectral-domain optical coherence tomography (Cirrus HD-OCT, Carl Zeiss Meditec, Dublin, CA). We defined the CFT as the distance between the inner retinal surface and the retinal pigment epithelium at the central fovea on the optical coherence tomography images and measured the CFT manually.

The VEGF concentration in the aqueous humor of eyes was measured by an enzyme-linked immunosorbent assay for human VEGF (R & D System, Minneapolis, MN). The primary antibody against VEGF detected 2 ($VEGF_{121}$ and $VEGF_{165}$) of the 4 VEGF isoforms.[8] The assay was performed according to the manufacturer's instructions. The lower limit of detection in this system was 31 pg/mL.

The data were analyzed using SigmaStat software (version 3.1, Systat Software Inc., Richmond, CA) and expressed as the mean ± standard deviation. The paired *t*-test was used to evaluate the difference in the VEGF concentrations in the aqueous humor samples before and after intravitreal injection of pegaptanib. The Wilcoxon signed rank test was used to evaluate the difference in the VEGF concentrations in the aqueous humor samples before and after intravitreal injection of ranibizumab. A P value of < 0.05 was considered statistically significant. The study was approved by the Institutional Review Board of Shiga University of Medical Science Hospital. All patients provided written informed consent before the start of the study.

Results

The VEGF concentrations in the aqueous humor of eyes with AMD ranged from 35.3 pg/mL to 142.4 pg/mL (mean ± standard deviation, 90.9 pg/mL ± 40.0 pg/mL) before the injection of pegaptanib and increased significantly ranging from 298.2 pg/mL to 571.3 pg/mL (mean ± standard deviation, 452.0 pg/ mL ± 106.4 pg/mL) 6 weeks after the first injection ($P = 0.005$; Figure 1A). The VEGF concentrations ranged from 47.2 pg/mL to 307.4 pg/mL (mean ± standard deviation, 125.9 pg/mL ± 77.2 pg/mL) before the injection of ranibizumab and decreased to <31 pg/mL, the lower limit of detection, 4 weeks after the first injection ($P < 0.001$; Figure 1B).

The visual acuity and CFT values are shown in Table 1. The mean logarithm of the minimum angle of resolution best-corrected visual acuity increased from 0.818 to 0.729 and the mean CFT decreased from 250.2 μm to 183.0 μm 6 weeks after an intravitreal injection of pegaptanib, although the VEGF concentration in the aqueous humor of eyes increased significantly after injection of pegaptanib. The mean logarithm of the minimum angle of resolution best-corrected visual acuity was maintained at the same level, 0.574 before injection and 0.627 4 weeks after injection of ranibizumab, whereas the mean CFT decreased from 307.9 μm to 237.3 μm 4 weeks after intravitreal injection of ranibizumab.

Discussion

We measured the VEGF concentration in the aqueous humor in patients with AMD before and

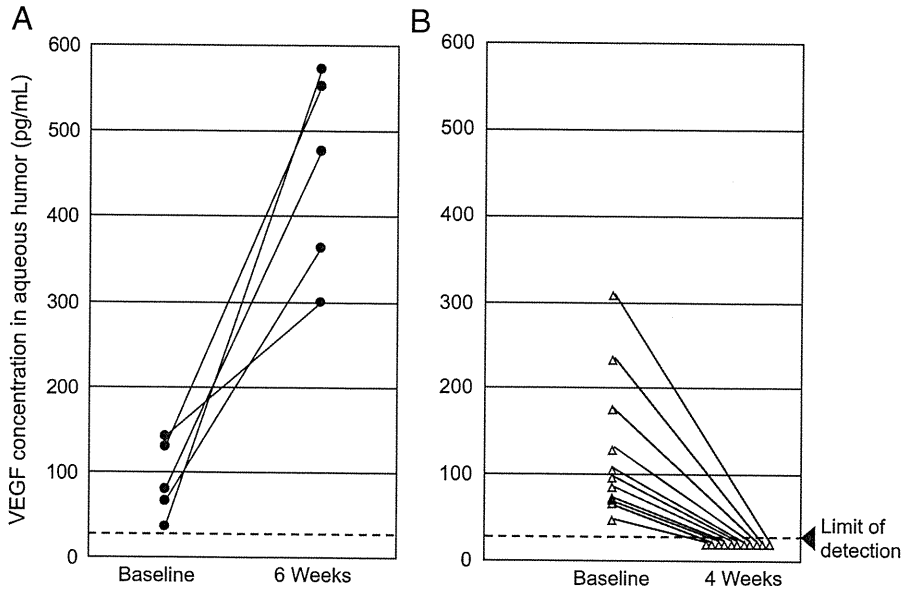


Fig. 1. A. Vascular endothelial growth factor concentrations in the aqueous humor before and after intravitreal injection of pegaptanib. The VEGF concentrations in the aqueous humor have increased significantly after intravitreal injections of 0.3 mg pegaptanib (paired *t*-test, $P = 0.005$). **B.** The VEGF concentrations in the aqueous humor before and after intravitreal injection of ranibizumab (Wilcoxon signed rank test, $P < 0.001$). The VEGF concentrations in the aqueous humor have decreased to an undetectable level after intravitreal injections of 0.5 mg ranibizumab.

after intravitreal injections of pegaptanib and ranibizumab and found that the VEGF concentration significantly decreased after injection of ranibizumab, as expected; however, unexpectedly, the VEGF concentration significantly increased after injection of pegaptanib.

Chan et al[14] reported that the aqueous VEGF levels decreased after intravitreal injection of bevacizumab in patients with CNV secondary to AMD. Because intravitreal bevacizumab decreased the elevated VEGF concentration to undetectable levels in the aqueous humor in patients with proliferative diabetic retinopathy,[13] it is reasonable that the VEGF concentration in the aqueous humor in patients with AMD would decrease to undetectable levels after intravitreal injection of bevacizumab. Ranibizumab has a higher affinity to VEGF than bevacizumab[15,16] and, therefore, it is also reasonable that intravitreal injection of ranibizumab would decrease the aqueous VEGF concentration similar to that after the injection of bevacizumab.

Table 1. Clinical Characteristics of Patients Before and After Intravitreal Injection of Anti-VEGF Agents

Case No.	Age (Years)	Sex	Anti-VEGF Drug	Aqueous VEGF Concentration (pg/mL) Before Treatment	Aqueous VEGF Concentration (pg/mL) After Treatment	Log MAR BCVA Before Treatment	LogMAR BCVA After Treatment	CFT Before Treatment (μm)	CFT After Treatment (μm)
1	84	M	Pegaptanib	66.2	362.4	0.52	0.82	213	116
2	69	M	Pegaptanib	35.3	571.3	1.22	1.05	284	273
3	78	M	Pegaptanib	80.5	475.5	0.82	0.52	132	197
4	61	M	Pegaptanib	142.4	298.2	0.22	0.10	400	173
5	84	M	Pegaptanib	130.2	552.5	1.30	1.15	222	156
6	74	M	Ranibizumab	72.0	<31	0.70	0.40	274	161
7	61	M	Ranibizumab	105.0	<31	0.70	0.82	468	320
8	76	M	Ranibizumab	65.8	<31	0.40	0.52	146	95
9	70	M	Ranibizumab	233.4	<31	1.05	1.00	502	631
10	73	F	Ranibizumab	128.9	<31	0.30	0.40	356	262
11	81	M	Ranibizumab	47.2	<31	0.70	1.05	217	221
12	87	M	Ranibizumab	95.2	<31	0.15	0.30	136	172
13	72	M	Ranibizumab	70.3	<31	0.40	0.52	275	212
14	72	M	Ranibizumab	85.4	<31	0.22	0.15	277	192
15	88	F	Ranibizumab	174.2	<31	0.70	0.82	330	123
16	95	M	Ranibizumab	307.4	<31	1.0	1.00	406	221

LogMAR, logarithm of the minimum angle of resolution; BCVA, best-corrected visual acuity; M, male; F, female.

Pegaptanib, unlike bevacizumab and ranibizumab, is a selective inhibitor of $VEGF_{165}$ with no affinity for other VEGF isoforms.[17] We expected $VEGF_{165}$ to decrease after intravitreal injection of pegaptanib, but we could not anticipate the behavior of the other VEGF isoforms. However, the VEGF concentration in the aqueous humor measured with an enzyme-linked immunosorbent assay surprisingly increased significantly after intravitreal injection of pegaptanib. Because the antibody used in the enzyme-linked immunosorbent assay detects $VEGF_{121}$ and $VEGF_{165}$, the total amount of $VEGF_{121}$ and $VEGF_{165}$, increases after the administration of pegaptanib. Because pegaptanib selectively inhibits $VEGF_{165}$, $VEGF_{165}$ was not expected to increase even 6 weeks after the injection. $VEGF_{121}$ was elevated 6 weeks after the injection, because each isoform such as transforming growth factor-β might react differently.[18] The involvement of $VEGF_{121}$ in CNV formation might be potential because it was reported that the increased expression of the $VEGF_{121}$ to $VEGF_{165-189}$ ratio resulted in the increased prostate tumor angiogenesis[19] and $VEGF_{121}$ recruited the peripheral vessels in tumors.[20] This might be why ranibizumab achieved better outcomes than pegaptanib.

In this study, the aqueous VEGF concentrations decreased in eyes treated with ranibizumab, whereas the concentrations increased in eyes treated with pegaptanib. However, an elevated aqueous VEGF concentration is not necessarily correlated with poor visual results. Indeed, the visual acuity improved and the CFT decreased after intravitreal injection of pegaptanib, although the aqueous VEGF concentration increased possibly because pegaptanib suppresses only $VEGF_{165}$, which is believed to be the pathogenic form of VEGF.[17,21] However, the visual acuity was maintained and the mean CFT decreased 4 weeks after intravitreal injection of ranibizumab, whereas the VEGF concentration in the aqueous humor decreased significantly after intravitreal injection of ranibizumab. The change in the visual acuity was not better 4 weeks after injection even in pivotal clinical studies.[8,9]

We found that the VEGF concentrations in the aqueous humor of eyes with AMD decreased after intravitreal injection of ranibizumab, but the VEGF concentration increased after intravitreal injection of pegaptanib. Further study, including specific measurement of the $VEGF_{121}$ and $VEGF_{165}$ isoforms, is needed to establish the pharmacokinetics of VEGF and anti-VEGF drugs.

Key words: age-related macular degeneration, anti-VEGF drug, aqueous humor, pegaptanib, ranibizumab, vascular endothelial growth factor.

References

1. Kvanta A, Algvere PV, Berglin L, Seregard S. Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. Invest Ophthalmol Vis Sci 1996;37:1929–1934.
2. Lopez PF, Sippy BD, Lambert HM, Thach AB, Hinton DR. Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. Invest Ophthalmol Vis Sci 1996;37:855–868.
3. Aiello AP, Pierce EA, Foley ED, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci USA 1995;92:10457–10461.
4. Adamis AP, Shima DT, Tolentino MJ, et al. Inhibition of vascular growth endothelial factor prevents retinal ischemia-associated iris neovascularization in a nonhuman primate. Arch Ophthalmol 1996;114:66–71.
5. Tobe T, Okamoto N, Vinores MA, et al. Evolution of neovascularization in mice with overexpression of vascular endothelial growth factor in photoreceptors. Invest Ophthalmol Vis Sci 1998;39:180–188.
6. Ozaki H, Seo MS, Ozaki K, et al. Blockade of vascular endothelial cell growth factor receptor signaling is sufficient to completely prevent retinal neovascularization. Am J Pathol 2000;156:697–707.
7. Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR; VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 2004;351:2805–2816.
8. Rosenfeld PJ, Brown DM, Heier JS, et al; MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med 2006;355:1419–1431.
9. Brown DM, Michels M, Kaiser PK, Heier JS, Sy JP, Ianchulev T; ANCHOR Study Group. Ranibizumab versus verteporfin photodynamic therapy for neovascular age-macular degeneration. Two-year results of the ANCHOR study. Ophthalmology 2009;116:57–65.e5.
10. Avery RL, Pieramici DJ, Rabena MD, Castellarin AA, Nasir MA, Giust MJ. Intravitreal bevacizumab (Avastin) for neovascular age-related macular degeneration. Ophthalmology 2006;113:363–372.
11. Spaide RF, Laud K, Fine HF, et al. Intravitreal bevacizumab treatment of choroidal neovascularization secondary to age-macular degeneration. Retina 2006;26:383–390.
12. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–2342.
13. Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M. Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. Arch Ophthalmol 2007;125:1363–1366.
14. Chan WM, Lai TY, Chan KP, et al. Changes in aqueous vascular endothelial growth factor and pigment epithelial-derived factor levels following intravitreal bevacizumab injections for choroidal neovascularization secondary to age-related macular degeneration or pathologic myopia. Retina 2008;28:1308–1313.
15. Presta LG, Chen H, O'Connor SJ, et al. Humanization of anti-vascular endothelial growth factor monoclonal antibody for the therapy of solid tumors and other disorders. Cancer Res 1997;57:4593–4599.
16. Chen Y, Wiesmann C, Fuh G, et al. Selection and analysis of an optimized anti-VEGF antibody: crystal structure of an

af-finity-matured Fab in complex with antigen. J Mol Biol 1999;293:865–881.
17. Ruckman J, Green LS, Beeson J, et al. 2′-Fluoropyrimidine RNA-based aptamers to the 165-amino acid form of vascular endothelial growth factor ($VEGF_{165}$): inhibition of receptor binding and VEGF-induced vascular permeability through interactions requiring the exon 7-encoded domain. J Biol Chem 1998;273:20556–20567.
18. Saed GM, Collins KL, Diamond MP. Transforming growth factors β_1, β_2 and β_3 and their receptors are differentially expressed in human peritoneal fibroblasts in response to hypoxia. Am J Reprod Immunol 2002;48:387–393.
19. Catena R, Muniz-Medina V, Moralejo B, et al. Increased expression of VEGF121/VEGF165-189 ratio results in a significant enhancement of human prostate tumor angiogenesis. Int J Cancer 2007;120:2096–2109.
20. Grunstein J, Masbad JJ, Hickey R, Giordano F, Johnson RS. Isoforms of vascular endothelial growth factor act in a coordinate fashion to recruit in expand tumor vasculature. Mol Cell Biol 2000;20:7282–7291.
21. Ishida S, Usui T, Yamashiro K, et al. $VEGF_{164}$-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. J Exp Med 2003;198:483–489.

Acta Ophthalmologica

EXTEND-I: safety and efficacy of ranibizumab in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration

Yasuo Tano[1] and Masahito Ohji[2] on behalf of the EXTEND-I Study Group*

[1]Department of Ophthalmology, Osaka University Medical School, Osaka, Japan
[2]Department of Ophthalmology, Shiga University of Medical Science, Shiga, Japan

ABSTRACT.

Purpose: **To evaluate the efficacy and safety of intravitreal ranibizumab for subfoveal choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD) in Japanese patients.**

Methods: **This open-label, multicentre, Phase I/II study enroled patients into Group A (single injection of ranibizumab nonrandomized doses of 0.3 or 0.5 mg followed by 11 monthly injections of the same dose) and Group B (12 monthly injections of ranibizumab randomized to 0.3 or 0.5 mg). The primary efficacy endpoint was the mean change from baseline in best-corrected visual acuity (BCVA) score at Month 6. Safety was evaluated in all patients who received ranibizumab.**

Results: **Of 88 patients enroled, 12 entered Group A (six per dose) and 76 entered Group B (0.3 mg: n = 35; 0.5 mg: n = 41). Mean change from baseline in BCVA was significantly increased for both doses (Group B) at Month 6 (0.3 mg: +8.1 letters, p = 0.0006; 0.5 mg: +9.0 letters, p < 0.0001) and Month 12 (0.3 mg: +9.5 letters, p = 0.0001; 0.5 mg: +10.5 letters, p < 0.0001). At Month 12, one patient (0.3 mg) and 0 patients (0.5 mg) lost ≥15 letters, while 37.1% (0.3 mg) and 31.7% (0.5 mg) of patients gained ≥15 letters. Ocular serious adverse events (SAEs) of the study eye were reported in 1 and 2 patients in the 0.3- and 0.5-mg groups, respectively. Nonocular SAEs were experienced by 2 and 5 patients in the 0.3- and 0.5-mg groups, respectively. No cases of endophthalmitis were reported.**

Conclusion: **Ranibizumab was effective and well tolerated in Japanese patients with subfoveal CNV secondary to AMD.**

Key words: age-related macular degeneration – best-corrected visual acuity score – choroidal neovascularization – efficacy – Japanese patients – ranibizumab – safety – subfoveal – tolerability

Acta Ophthalmol.

doi: 10.1111/j.1755-3768.2009.01843.x

*The EXTEND-I Study Group: Tomohiro Iida (Fukushima Medical University Hospital), Tatsuro Ishibashi (Kyushu University Hospital), Susumu Ishida (Keio University Hospital), Shoji Kishi (Gunma University Faculty of Medicine), Yuichiro Ogura (Nagoya City University Hospital), Masahito Ohji (Shiga University of Medical Science Hospital), Annabelle A Okada (Kyorin University Hospital), Yukihiro Sato (Toho University Sakura Hospital), Fumio Shiraga (Kagawa University Hospital), Kunihiko Shiraki (Osaka City University Medical School Hospital), Yasuo Tano (Osaka University Medical School), Hiroko Terasaki (Nagoya University Hospital), Fumihiko Yagi (Toho University Ohashi Medical Center), Nagahisa Yoshimura (Kyoto University Hospital) and Mitsuko Yuzawa (Surugadai Nihon University Hospital).

Introduction

Age-related macular degeneration (AMD) is a significant health problem. In particular, neovascular AMD is a progressive retinal disease that can cause severe and irreversible vision loss and can lead to legal blindness if left untreated (Nowak 2006). Furthermore, neovascular AMD can cause severe emotional distress and have a profound impact on patients' quality of life (Hassell et al. 2006; Augustin et al. 2007).

According to findings of the Hisayama study (prospective cohort study in Japan), the prevalence of neovascular AMD in residents aged 50 years or older (n = 1486) was 0.67% (1.2% in men, 0.34% in women) in 1998, which was lower than that observed in Caucasians (Oshima et al. 2001). However, another recent study (the Funagata study) of Japanese residents aged 35 years or older (n = 1758) between 2000 and 2002 suggested that although the prevalence of neovascular AMD was lower in Japanese women, it was similar to that seen in Caucasians for Japanese men (Kawasaki et al. 2008).

Until recently, available therapies for neovascular AMD in Japan were laser photocoagulation and photodynamic therapy (PDT) with verteporfin. Pegaptanib sodium was launched in 2008, and ranibizumab was launched

in early 2009 in Japan. Several studies have demonstrated the long-term safety and efficacy of verteporfin PDT in Japanese patients with AMD (Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2003, 2008); however, there is still little experience of pegaptanib sodium and ranibizumab in Japanese patients.

Ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland and Genentech, Inc., South San Francisco, CA, USA) is a Fab fragment of a recombinant, humanized, monoclonal antibody. Ranibizumab specifically binds to and inhibits all biologically active isoforms of vascular endothelial growth factor-A (VEGF), thus blocking vascular permeability and angiogenesis in neovascular AMD (Ferrara et al. 2006; Dadgostar & Waheed 2008). Two pivotal Phase III clinical studies (ANCHOR and MARINA) have demonstrated unprecedented good efficacy and acceptable safety profiles for ranibizumab in patients with neovascular AMD (Brown et al. 2006; Rosenfeld et al. 2006), leading to ranibizumab being licensed for neovascular AMD in the United States by the Food and Drug Administration in 2006 (FDA 2006) and in the European Union in 2007.

The ANCHOR and MARINA studies were conducted in populations comprising predominantly Caucasian patients. EXTEND-I is the first clinical study to investigate the efficacy and safety of intravitreal ranibizumab specifically in Japanese patients with subfoveal choroidal neovascularization (CNV) secondary to AMD. This report describes the 12-month safety of single and multiple dosing and efficacy of multiple dosing of ranibizumab from the EXTEND-I study.

Methods

Study objectives

The primary objectives of the EXTEND-I study were to evaluate the safety of intravitreal administration of ranibizumab as single or multiple doses and to assess the efficacy of ranibizumab multiple dosing for 6 months. The secondary objectives were to compare the efficacy of multiple doses of ranibizumab (also at Month 12) in Japanese patients with that observed in previous non-Japanese studies and to characterize the pharmacokinetics of intravitreal ranibizumab in Japanese patients.

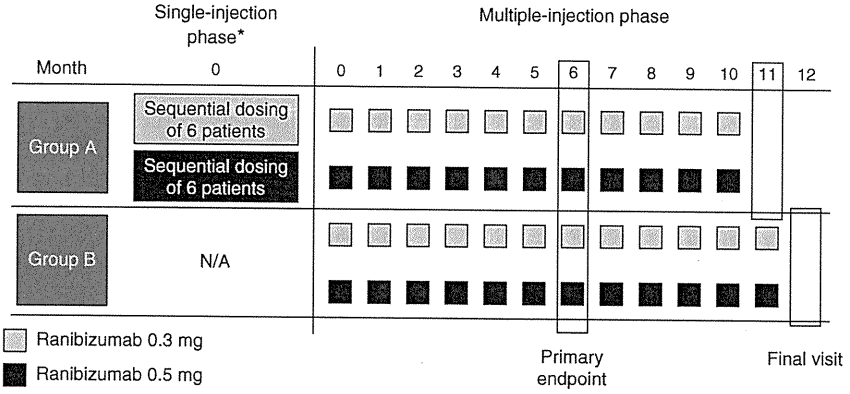
EXTEND-I study design

This was an open-label, multicentre, Phase I/II study comprising two phases (a single-injection phase and a multiple-injection phase) and two groups of patients (Groups A and B) (Fig. 1). In the single-injection phase, the safety of single intravitreal injections of ranibizumab (not randomized, doses of 0.3 or 0.5 mg) was evaluated in sequential cohorts of two patients in Group A. Those patients in Group A who successfully completed the single-injection phase [i.e. did not experience a grade-3 targeted adverse event (AE)] could enter a multiple-injection phase whereby they received monthly injections of ranibizumab for an additional 11 months at the same dose as they received in the single-injection phase. This multiple-injection phase was also initiated in a population of patients classified as Group B. In the multiple-injection phase, Group-B patients were randomized equally to receive a total of 12 monthly intravitreal injections of ranibizumab at doses of 0.3 or 0.5 mg. Both safety and efficacy were evaluated in Group B.

The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice, and was approved by the Institutional Review Board at each site. All patients provided written, informed consent before determination of their full eligibility.

Patients

Male or female patients aged ≥50 years with primary or recurrent subfoveal CNV secondary to AMD (including patients with predominantly classic lesions, minimally classic lesions or occult lesions with no classic component in a ratio of 1:1:1) were enroled from 15 study sites in Japan. Other inclusion criteria were a total area of CNV (including both classic and occult components) ≥50% of the total lesion area, total lesion size ≤5400 μm in the greatest linear dimension and a best-corrected visual acuity (BCVA) score between 73 and 24 letters in the study eye (approximate Snellen equivalent of 20/40 to 20/320), assessed with the use of Early Treatment Diabetic Retinopathy Study (ETDRS) charts. Patients were excluded if they had a BCVA score of < 34 letters in both eyes, had previously participated in a clinical study involving antiangiogenic drugs (for either eye), or had participated in a clinical study of any investigational drugs (excluding vitamins and minerals) within 1 month preceding EXTEND-I study commencement. Patients were also excluded if they had received previous treatment in the study eye with PDT with verteporfin, radiation therapy, macular laser photocoagulation, vitrectomy or transpupillary thermotherapy, or had a subretinal haemorrhage in the study eye involving the centre of the fovea with a size of either ≥50% of the total lesion area or ≥1 disc area (DA) in size.



*Upon completion of the single-injection phase, patients in Group A were eligible to enter the multiple-injection phase, which began ≥4 weeks after the final visit of the single-injection phase.
Multiple injections did not begin until both doses were shown to be well tolerated in all cohorts

Fig. 1. EXTEND-I study design.

Assessments

Efficacy

The primary efficacy variable was the mean change from baseline in BCVA score, assessed with the use of ETDRS charts at a starting distance of 2 m, in the study eye at Month 6 for both ranibizumab doses; the mean change from baseline in BCVA score at Month 12 was evaluated as a secondary variable. Other secondary efficacy variables included the assessment at Months 3, 6, 9 and 12 of the total area of CNV, the total area of leakage from CNV plus staining of the retinal pigment epithelium (RPE), and the proportion of patients with absence of leakage from CNV. Total area of CNV and total area of leakage from CNV plus RPE staining were measured in the study eye by fluorescein angiography (FA). In addition, the proportions of patients at Months 6 and 12 with a BCVA score loss in the study eye of <15 letters, ≥ 15 letters and ≥ 30 letters, a BCVA score gain of ≥ 15 letters, and a BCVA score of <34 letters (approximate Snellen equivalent of 20/200) were evaluated. Snellen equivalents were determined with the ETDRS chart at a starting distance of 2 m. The change and per cent change from baseline in foveal retinal thickness at Months 3, 6, 9 and 12 were also investigated in study eyes using optical coherence tomography (OCT). The OCT imaging was performed using OCT 3000 (Stratus OCT™; Carl Zeiss Meditec AG, Jena, Germany) with software version A1.1 or later. OCT operators, systems and software were certified by the reading centre prior to the enrolment of study patients. Similarly for FA, photographers were also certified by the reading centre. FA and OCT images were interpreted at a designated central reading centre, the University of Wisconsin Fundus Photograph Reading Center (Madison, WI, USA).

Pharmacokinetics

Pharmacokinetic analysis was conducted in the single-injection phase of Group A. Blood samples were taken from all 12 patients in Group A at six time-points (1 hr before and 2 hr after ranibizumab single injection at baseline, and 24 hr, 3, 7 and 14 days after single injection). Serum ranibizumab concentration was assayed by Genentech, Inc. The pharmacokinetics of serum ranibizumab was analysed by noncompartmental methods (using WinNonlin Pro, Version 5: Pharsight, St Louis, MO, USA) and the pharmacokinetic parameters were summarized for each dose group. Area under the curve (AUC) was measured from time 0 to the last measurable time-point.

Safety

The primary safety variable was the incidence of grade-3 targeted AEs up to Month 6; targeted AEs were assessed in both the study and fellow eye consisted of intraocular inflammation (with grade-3 defined as any 4+ intraocular inflammation or 2–3+ intraocular inflammation that fails to decrease to $\leq 1+$ within 30 days) (Hogan et al. 1959), decreases in VA (with grade-3 defined as >29-letter decrease within 14 days after ranibizumab administration compared with before administration), retinal tear or detachment (with grade-3 defined as a new tear or detachment developing during the study and involving the macula), retinal haemorrhage (with grade-3 defined as any new haemorrhage >1 DA in size and involving the fovea, or an increase of a pre-existing haemorrhage by >1 DA and involving the fovea), vitreous haemorrhage (with grade-3 defined as any vitreous haemorrhage of $\geq 2 \pm$ severity lasting >14 days), and increases or decreases in intraocular pressure [with grade-3 defined as a persistent (>15 min) loss of light perception because of increased intraocular pressure, or a >20 mmHg increase or decrease in intraocular pressure lasting ≥ 14 days].

Serious adverse events (SAEs) were identified for special reporting requirement. Eye-related AEs were assessed by nondirective questioning and ophthalmic examinations; other AEs were detected by nondirective questioning, vital signs, laboratory values or other assessments. Pregnancy testing (urine) was performed on female patients (of child-bearing potential) at the screening visit. Serum samples for the evaluation of immunoreactivity to ranibizumab (antiranibizumab antibodies) were obtained from all patients prior to the first study drug administration, and from patients who had been treated with multiple doses of ranibizumab for ≥ 6 months at Month 6 in both Groups A and B, at Month 11 in Group A and at Month 12 in Group B.

Haematology, serum chemistry, urinalysis and vital signs were monitored regularly, and all AEs were collected and evaluated for their severity and relationship to the study drug.

Statistical analyses

For both Group A and Group B patients, demographic characteristics and baseline ocular characteristics were summarized for the enroled population (all enroled patients). The discrete variables were presented as the number and percentage of patients in each category, and the continuous variables were summarized using descriptive statistics (mean, median, standard deviation and range). Safety analyses, including drug exposure, were conducted in the safety population (all enroled patients who received at least one dose of the study drug and had at least one postbaseline safety assessment) in Groups A and B. Efficacy was not analysed in Group A.

For Group B, efficacy analyses were performed for three different populations: intent-to treat (ITT) population, per protocol population (PP) and patients with at least one measurement of OCT. The analysis of the primary efficacy variable was performed on the study eye in the ITT population using the last observation carried forward method to impute any missing data. In addition, to assess the robustness of the data, analysis of the primary efficacy variable was repeated on the PP population and the ITT population with observed data. Descriptive statistics for the change in BCVA score from baseline were summarized by treatment and by visit. The 95% confidence intervals (95% CI) for the change in BCVA score from baseline were based on *t*-distributions, and the p-values were based on paired *t*-tests.

Subgroup analyses were performed for the mean change from baseline in BCVA score at Months 6 and 12 by CNV lesion classification (predominantly classic, minimally classic and occult with no classic component), age (<75, ≥ 75 years), gender, baseline BCVA score in study eye (<55, ≥ 55; <45, ≥ 45 letters) and lesion size (≤ 2, 2–4 DA; >4 DA).

Analyses of the secondary efficacy variables were performed using the

same approaches as for the primary efficacy variable, with the exception of foveal retinal thickness by OCT, which was only analysed for the ITT population with observed data. These 12-month analyses of Group A and Group B data were based on data cut-offs at Month-11 and Month-12 visits for each patient, respectively, in the multiple-injection phase.

Results

Patients

Overall, 88 patients were enroled in the study: 12 in Group A (six per dose) and 76 in Group B (35 in the 0.3-mg-dose group and 41 in the 0.5-mg-dose group). Patient demographics and baseline characteristics are shown in Table 1.

In Group A, 12 patients completed the single-injection phase; of these, 11 patients subsequently entered the multiple-injection phase (one patient in the 0.3-mg-dose group chose to receive other therapy instead of entering the multiple-injection phase). Overall, 10 patients from Group A completed the multiple-injection phase; one patient in the 0.3-mg-dose group withdrew from the study because of an AE.

In Group B, eight of the 76 patients discontinued from the study prematurely; four in each of the ranibizumab 0.3-mg-dose group and 0.5-mg dose group. Discontinuations from the study were because of death (n = 2, one in each dose group), AEs (n = 2, one in each dose group), protocol violation (n = 1 in the 0.3-mg-dose group) and withdrawn consent (n = 3, one in the 0.3-mg-dose group and two in the 0.5 mg-dose group). None of the events leading to study discontinuation or death was thought to be related to the study treatment.

Efficacy (multiple-injection phase of Group B)

A significant increase in mean BCVA score in the study eye (standard deviation, SD), which was the primary efficacy endpoint, was observed between baseline (47.6 letters in the 0.3 mg and 48.1 letters in the 0.5 mg) and Month 6: +8.1 (12.65) letters (p = 0.0006, paired t-test) in the 0.3-mg-dose group and +9.0 (9.62) letters (p < 0.0001, paired t-test) in the 0.5-mg-dose group. Increases in mean BCVA score from baseline were seen after 1 month of ranibizumab treatment, namely increases of +5.2 (9.19) letters in the 0.3-mg-dose group and +5.8 (7.45) letters in the 0.5-mg-dose group. The improved BCVA scores at Month 6 were maintained up to Month 12, where increases of +9.5 (12.79) letters (p = 0.0001, paired t-test) in the 0.3-mg-dose group and +10.5 (11.4) letters (p < 0.0001, paired t-test) in the 0.5-mg-dose group were observed (Fig. 2).

At Month 6, the number of patients in the 0.3-mg-dose group and 0.5-mg-dose group who lost ≥15 letters in BCVA score in the study eye was 1 and 0, respectively, while the proportion of patients who gained ≥15 letters in BCVA score in the study eye was 34.3% and 24.4%, respectively. At Month 6, the proportion of patients in the 0.3-mg-dose group and 0.5-mg-dose group who had a BCVA of the approximate Snellen equivalent of 20/40 or better in the study eye was 11.4% and 29.3%, respectively, while the proportion of patients having a BCVA of the approximate Snellen equivalent of 20/200 or worse in the study eye at this time-point was 14.3% and 7.3%, respectively. Improvements in these secondary efficacy variables of

Table 1. Patient demographics and baseline characteristics.

	Group A		Group B	
	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
Gender (% male)	83.3	83.3	74.3	80.5
Age (mean years)	70.3	72.0	70.7	71.6
Mean BCVA letters (SD)	52.0 (16.90)	44.2 (14.95)	47.6 (11.82)	48.1 (10.75)
BCVA (median Snellen equivalent)	20/80	20/200	20/125	20/125
CNV classification (%)				
Occult with no classic	–	–	40.0	34.1
Minimally classic	–	–	34.3	41.5
Predominantly classic	–	–	25.7	24.4
Total area of lesion (DA)	–	–	2.35	2.36

BCVA = best-corrected visual acuity; CNV = choroidal neovascularization; DA = disc areas; SD = standard deviation.

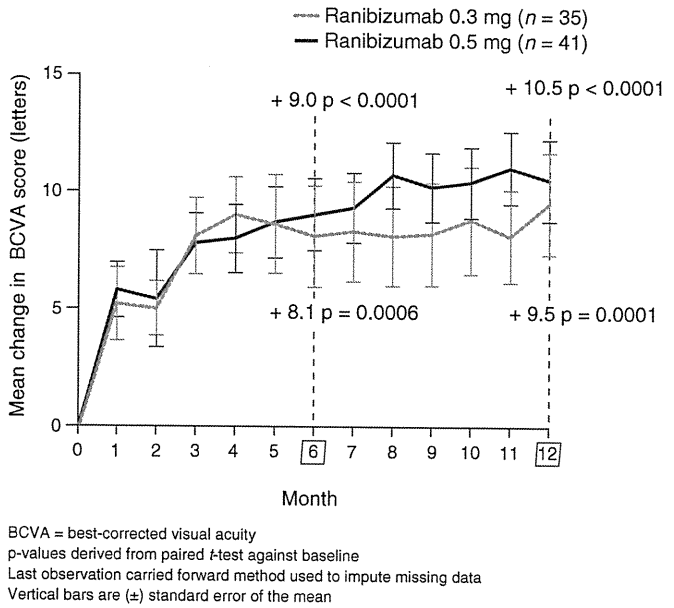


Fig. 2. Mean change from baseline in best-corrected visual acuity score with Early Treatment Diabetic Retinopathy Study chart over time in the study eye of multiple injection phase in Group B.

vision were also seen at Month 12 in both dose groups (Fig. 3).

The total area of CNV remained constant throughout 12 months in both dose groups. The mean change (SD) from baseline at Months 3, 6, 9 and 12 was −0.10 (0.95), −0.15 (0.97), −0.23 (0.97) and −0.16 (1.01) DA, respectively, in the 0.3-mg-dose group, and 0.13 (0.75), 0.04 (0.76), 0.21 (0.90) and 0.23 (1.08) DA, respectively, in the 0.5-mg-dose group.

Both dose groups showed a statistically significant decrease from baseline in the total area of leakage from CNV plus RPE staining and foveal retinal thickness over time. The total area of leakage from CNV plus RPE staining at Month 12 decreased by more than half of that at baseline; mean change (SD) of −1.50 (1.08) DA at Month 12 from 2.31 (1.17) DA at baseline ($p < 0.0001$, paired t-test) in the 0.3-mg-dose group, and −1.39 (1.48) DA at Month 12 from 2.49 (1.54) DA at baseline ($p < 0.0001$, paired t-test) in the 0.5-mg-dose group. At Month 12, foveal retinal thickness was significantly reduced compared with baseline in both ranibizumab-dose groups: the mean percentage change in the 0.3-mg-dose group was −41.6% (95% CI of −57.5, −25.6; $p < 0.0001$, paired t-test) and in the 0.5-mg-dose group, it was −58.9% (95% of CI −71.1, −46.7; $p < 0.0001$, paired t-test) (Fig. 4).

There was an increase in mean BCVA score at Month 12 in all analysed subgroups, with the exception of the subgroup of patients with a BCVA score of ≥55 letters at baseline in the 0.3-mg-dose group ($n = 10$, mean change −0.1 letters, SD 12.18 letters).

Pharmacokinetics

Pharmacokinetic data for each ranibizumab dose are shown in Table 2. Ranibizumab showed a slow systemic absorption with a mean T_{max} of 0.53 and 1.00 days for the 0.3-mg-dose group and 0.5-mg-dose group, respectively. Apparent mean $t_{½}$ in the 0.3-mg-dose group and 0.5-mg-dose group, respectively, was 6.56 and 7.85 days.

Safety

All 88 enroled patients received at least one dose of ranibizumab and had at least one postbaseline safety assessment: 12 in Group A; single and multiple-injection phase (six per dose group) and 76 in Group B; multiple-injection phase (35 in the 0.3-mg-dose group and 41 in the 0.5-mg-dose group). Table 3 summarizes the exposure of patients to ranibizumab treatment, including mean treatment duration. For patients in Group A, the treatment duration includes the transitional interval between the single- and multiple-injection phases. The length of this interval between the first and second injections varied for each patient. The maximum number of injections in both the groups was 12. Overall, 89% (78/88) of patients in pooled Groups A and B received >9 injections of ranibizumab.

No patients in Group A experienced a grade-3 targeted AE. However, a grade-3 targeted AE of 'retinal haemorrhage' in the study eye was

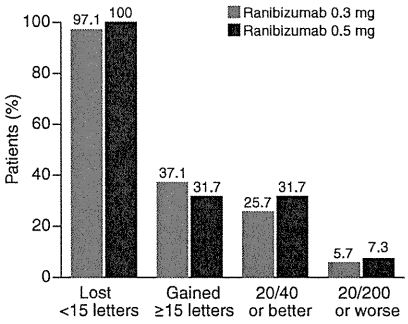
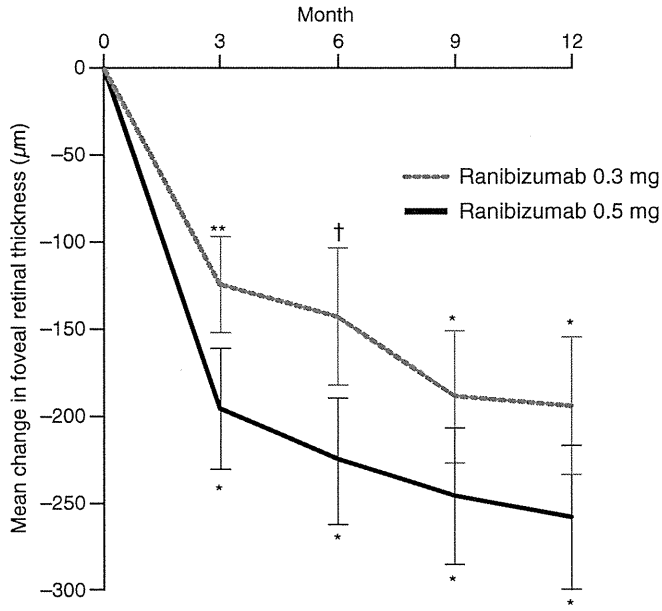


Fig. 3. Proportion of patients who lost <15 letters, gained ≥15 letters, had an approximate Snellen equivalent of 20/40 or better or had an approximate Snellen equivalent of 20/200 or worse in best-corrected visual acuity with Early Treatment Diabetic Retinopathy Study chart at Month 12 in the study eye of multiple injection phase in Group B.



*p < 0.0001; ** p = 0.0001; †p = 0.0012

Error bars are ±1 standard error of the mean

p -values derived from paired t-test against baseline

The numbers of patients in the 0.3 and 0.5 mg dose groups, respectively, were as follows:

Baseline; $n = 28$, $n = 30$

Month 3; $n = 27$, $n = 29$

Month 6; $n = 26$, $n = 28$

Month 9; $n = 26$, $n = 27$

Month 12; $n = 26$, $n = 26$

Fig. 4. Mean change from baseline in foveal retinal thickness in the study eye of multiple injection phase in Group B.

experienced by one patient (2.4%) in the 0.5-mg-dose group of Group B during the first 6 months of the multiple-injection phase. The overall incidence of grade-3 targeted AEs at Month 6 (the primary safety endpoint) was 1.3% (n = 1) of 76 patients in Group B. Additionally, grade-3-targeted AEs of 'visual acuity reduced transiently' in the study eye were experienced by two patients (4.9%) in the 0.5-mg-dose group of Group B immediately after an accidental overdose of ranibizumab (approximately 0.2–0.3 ml). The overall incidence of grade-3-targeted AEs at Month 12 was 3.9% (n = 3) in Group B. Approximately 90% of patients experienced at least one ocular AE in the study eye during the 12-month study period; namely, 94.3% (n = 33 of 35) of patients in the 0.3-mg-dose group and 82.9% (n = 34 of 41) of patients in the 0.5-mg-dose group of Group B. The most common AE of the study eye was 'conjunctival haemorrhage' (74.3% and 58.5% of patients in the 0.3 mg and 0.5-mg-dose groups of Group B, respectively), most of which were thought to be associated with the intravitreal injection procedure. No endophthalmitis was observed throughout the study.

Ocular AEs in the study eye suspected to be related to the study drug were experienced by two patients in the 0.5-mg-dose group of Group A (increased intraocular pressure, n = 1; decreased visual acuity, n = 1), and by 6 (17.1%) patients in the 0.3 mg dose of Group B and 10 (24.4%) patients in the 0.5-mg-dose group of Group B (Table 4). The most common ocular AE in the study eye in Group B was 'intraocular pressure increased' (5.7% in the 0.3 mg dose; 12.2% in the 0.5 mg dose). Furthermore, two patients in the 0.5-mg-dose group of Group B who received an accidental overdose of ranibizumab experienced the following ocular AEs: 'intraocular pressure increased' (study eye, both patients), 'visual acuity reduced transiently' (study eye, both patients), 'eye pain' (study eye, one patient), 'corneal oedema' (study eye, one patient) and 'asthenopia' (study eye, one patient).

Adverse events of intraocular inflammation were not observed in Group A. 'Anterior chamber inflammation' and 'iritis' of the study eye were observed in one patient each in the 0.3-mg-dose group of Group B. 'Anterior chamber inflammation' of the fellow eye was experienced by one patient in the 0.3-mg-dose group and 'iritis' of the fellow eye was experienced by one patient in each of the 0.3-mg-dose group and 0.5-mg-dose group of Group B.

Table 2. Pharmacokinetic parameters for ranibizumab after single administration.

Pharmacokinetic parameter	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)
T_{max}, mean (range), days	0.53 (0.08–3.02)	1.00 (0.97–2.97)
C_{max}, mean (SD), ng/ml	1.96 (1.65)	1.86 (0.61)
$AUC_{0\text{-}T}$, mean (SD), ng* day/ml	7.47 (3.98)	14.90 (2.86)
$T_{½}$, mean (SD), days	6.56 (3.85)	7.85 (3.38)

$AUC_{0\text{-}T}$ = area under the curve (time 0 to last measurable time-point); C_{max} = highest systemic drug level; SD = standard deviation; $T_{½}$ = serum elimination half-life; T_{max} = time to achieve the highest systemic drug level.

Table 3. Summary of patient exposure to ranibizumab.

	Group A		Group B	
	Ranibizumab 0.3 mg (n = 6)	Ranibizumab 0.5 mg (n = 6)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
No. of injections, mean (range)	9.3 (1–12)	12.0 (12–12)	11.2 (3–12)	11.1 (3–12)
<3	1	0	0	0
3–6	0	0	3	4
>6–9	1	0	1	0
>9–12	4	6	31	37
Treatment duration (days) mean (range)	382.2 (1–578)	417.8 (345–463)	306.6 (66–337)	305.3 (57–337)
Treatment interval from first to second injection (days) mean (range)	180.2 (134–281)	118.7 (51–166)	–	–

Table 4. Summary of study drug-related ocular and nonocular adverse events (AEs) (Group B patients).

AE, n (%)	Ranibizumab 0.3 mg (n = 35)	Ranibizumab 0.5 mg (n = 41)
Ocular AEs		
Total	6 (17.1)	10 (24.4)
Intraocular pressure increased	2 (5.7)	5 (12.2)
Eye pain	0	3 (7.3)
Visual acuity reduced transiently	0	2 (4.9)
Anterior chamber inflammation	1 (2.9)	0
Conjunctival hyperaemia	1 (2.9)	0
Conjunctival oedema	1 (2.9)	0
Retinal haemorrhage	1 (2.9)	0
Visual acuity reduced	1 (2.9)	1 (2.4)
Asthenopia	0	1 (2.4)
Corneal oedema	0	1 (2.4)
Lymphangiectasia	0	1 (2.4)
Posterior capsule opacification	0	1 (2.4)
Vitreous floaters	0	1 (2.4)
Nonocular AEs		
Total	0	3 (7.3)
Angina pectoris	0	1 (2.4)
Eczema	0	1 (2.4)
Hypertension	0	1 (2.4)

Table 5. Deaths and serious adverse events (SAEs) during the study period.

	Group A Ranibizumab 0.3 mg $n = 6$	Group A Ranibizumab 0.5 mg $n = 6$	Group B Ranibizumab 0.3 mg $n = 35$	Group B Ranibizumab 0.5 mg $n = 41$
Death	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.4)
Total SAEs	1 (16.7)	0 (0.0)	4 (11.4)	8 (19.5)
Ocular SAE of study eye	0 (0.0)	0 (0.0)	1 (2.9)	2 (4.9)
Corneal oedema	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Eye pain	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Intraocular pressure increased	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.9)
Visual acuity reduced	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Visual acuity reduced transiently	0 (0.0)	0 (0.0)	0 (0.0)	2 (4.9)
Ocular SAE of fellow eye	0 (0.0)	0 (0.0)	2 (5.7)	1 (2.4)
Cataract	0 (0.0)	0 (0.0)	1 (2.9)	1 (2.4)
Macular degeneration	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Nonocular SAE	1 (16.7)	0 (0.0)	1 (2.9)	5 (12.2)
Angina pectoris	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Anorexia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Ataxia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Bladder neoplasm	0 (0.0)	0 (0.0)	1 (2.9)	0 (0.0)
Cerebral haemorrhage	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Diabetes mellitus	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
Gastric cancer	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Hypoesthesia	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)
Lung carcinoma cell type unspecified recurrent	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)

Nonocular AEs suspected to be related to ranibizumab were reported by one patient in the 0.3-mg-dose group of Group A (intestinal diverticulum), and three patients in the 0.5-mg-dose group of Group B (Table 4).

There were 13 SAEs and two deaths in this study (Table 5). In Group A, one nonocular SAE of 'diabetes mellitus' was experienced by one patient (0.3-mg-dose group) during the multiple-injection phase. In Group B, ocular (study and fellow eye) or nonocular SAEs were experienced by four patients in the 0.3-mg-dose group and eight patients in the 0.5-mg-dose group. The SAEs in the Group B were 'corneal oedema', 'eye pain', 'intraocular pressure increased', 'visual acuity reduced' 'visual acuity reduced transiently' (accidental overdose of ranibizumab),'cataract', 'macular degeneration', 'angina pectoris', 'bladder neoplasm', 'cerebral haemorrhage', 'gastric cancer' and 'lung carcinoma cell type unspecified recurrent'. One patient experienced 'anorexia', 'ataxia' and 'hypoesthesia'. 'Bladder neoplasm' (0.3 mg Group B) resulted in the death of a patient during the first 6 months of the multiple-injection phase. There was another death (0.5-mg Group B) that resulted from a nonocular SAE of 'lung carcinoma cell type unspecified recurrent'. Neither of the deaths was suspected to be related to the study drug.

The study was discontinued by five patients because of nonocular AEs. This includes the two patients who died, two patients who reported an SAE and one patient in Group A (0.3-mg-dose group; 'vomiting' because of Fluorescein injection at Month 6).

The analyses of AEs potentially related to systemic VEGF inhibition focused on the incidence of hypertension, arterial thromboembolic events and nonocular haemorrhage. Overall, no such AEs were observed in the Group-A patients. In Group-B patients, they were observed in three of 35 patients (8.6%) in the 0.3-mg-dose group and in three of 41 patients (7.3%) in the 0.5-mg-dose group. 'Angina pectoris' and 'cerebral haemorrhage' were experienced by one patient (2.4%) each in the 0.5-mg-dose group. 'Hypertension' was experienced by three patients (8.6%) in the 0.3-mg-dose group and one patient (2.4%) in the 0.5-mg-dose group. Stroke ('cerebral haemorrhage') was observed in one patient (2.4%) in the 0.5-mg-dose group. Of these events, 'angina pectoris' and 'hypertension' in the 0.5-mg-dose group were suspected to be related to the study drug.

At Month 11 in the multiple-injection phase of Group A, immunoreactivity to ranibizumab (antiranibizumab antibodies) was not detected in any patient. At Month 12 in Group B, immunoreactivity to ranibizumab was detected in one of 32 evaluated patients (3.1%) in the 0.3-mg-dose group and three of 37 evaluated patients (8.1%) in the 0.5-mg-dose group. However, none of these patients had any AEs suspected to be related to the study drug.

Discussion

The results reported in this study demonstrate that monthly intravitreal ranibizumab significantly improves VA, FA and OCT outcomes in Japanese patients with subfoveal CNV secondary to AMD. Ranibizumab significantly increased the mean BCVA score from baseline to Month 6 with both 0.3 mg (+8.1 letters) and 0.5 mg (+9.0 letters) doses. The improved BCVA scores persisted to Month 12 in both the 0.3-mg-dose group (+9.5 letters) and 0.5-mg-dose group (+10.5 letters).

In addition, compared with baseline, ranibizumab significantly reduced the total area of leakage from CNV plus RPE staining and foveal retinal thickness.

The results in this study are consistent with those previously reported in the pivotal Phase III studies (MARINA and ANCHOR) conducted in a predominantly Caucasian population of patients with neovascular AMD (Brown et al. 2006; Rosenfeld et al. 2006). These studies showed an increase from baseline in mean BCVA score at 12 months of 6.5–8.5 letters with ranibizumab 0.3 mg and 7.2–11.3 letters with ranibizumab 0.5 mg, compared with decreases in mean BCVA score of 10.4 letters observed with sham treatment in the MARINA study and 9.5 letters with verteporfin PDT in the ANCHOR study. In addition, ≥97% of patients in this study lost fewer than 15 letters after 12 months of ranibizumab treatment, which is also similar to that seen in the MARINA and ANCHOR studies (approximately 95%). The proportions of patients who gained ≥15 letters were also similar among these three studies (approximately 30–40%).

The improvement in BCVA score observed with ranibizumab in this study and large-scale randomized double-masked clinical studies may reflect the ability of ranibizumab to inhibit all diffusible isoforms of VEGF that are biologically active, specifically $VEGF_{165}$, $VEGF_{121}$ and $VEGF_{110}$ (Lowe et al. 2007).

The calculated $t_{½}$ corresponded with the absorption rate of ranibizumab from the eye into the systemic circulation as a result of flip-flop pharmacokinetics associated with sustained release, and suggested a low elimination rate of ranibizumab from the eye. These pharmacokinetic findings in Japanese patients are consistent with those in non-Japanese patients described in the prescribing information of Lucentis (FDA, 2006).

Intravitreal ranibizumab treatment was associated with an acceptable safety and tolerability profile in this Japanese patient population. The most common AE was conjunctival haemorrhage of mild severity, most of which was thought to be because of the intravitreal injection procedure. Notably, there were no incidences of endophthalmitis in this study, which has also been mainly attributed to an intravitreal injection procedure. Among the observed ocular and nonocular SAEs, with the exception of overdose-related SAEs, only one SAE of angina pectoris in the 0.5-mg-dose group of Group B was suspected to be related to the study drug. The incidence of grade-3 targeted AEs in Group B was 1.3% (n = 1 of 35) at Month 6 and 3.9% (n = 3 of 41) at Month 12 in Group B. In addition, the incidences of nonocular AEs suspected to be related to ranibizumab and AEs potentially related to systemic VEGF inhibition were also within the acceptable range and consistent with the earlier studies (Brown et al. 2006; Rosenfeld et al. 2006).

In conclusion, the results of this study are comparable with previous randomized double-masked Phase III studies in predominantly Caucasian patients and indicate that monthly intravitreal ranibizumab therapy with 0.3 and 0.5 mg doses has an acceptable safe profile and is highly effective in Japanese patients with subfoveal CNV secondary to neovascular AMD.

In Japanese patients, clinically and statistically significant improvements in mean BCVA score of approximately two lines on the ETDRS chart have been achieved during 12 months of monthly treatment with ranibizumab.

Acknowledgements

The authors acknowledge former principal investigator, Dr Shinobu Takeuchi (Toho University Ohashi Medical Center), and Dr Takashi Tokoro (Tokyo Medical and Dental University) for their contribution to the study protocol and clinical study report.

The authors acknowledge medical writing assistance from Vanessa Cobb from Complete Medical Communications. This study was funded unconditionally by Novartis. The views and opinions expressed herein are those of the authors.

The EXTEND-I study was presented at the ARVO 2008 annual meeting as a poster presentation.

References

Augustin A, Sahel JA, Bandello F, Dardennes R, Maurel F, Negrini C, Hieke K & Berdeaux G (2007): Anxiety and depression prevalence rates in age-related macular degeneration. Invest Ophthalmol Vis Sci **48**: 1498–1503.

Brown DM, Kaiser PK, Michels M, Soubrane G, Heier JS, Kim RY, Sy JP & Schneider S (2006): Ranibizumab versus verteporfin for neovascular age-related macular degeneration. N Engl J Med **355**: 1432–1444.

Dadgostar H & Waheed N (2008): The evolving role of vascular endothelial growth factor inhibitors in the treatment of neovascular age-related macular degeneration. Eye **22**: 761–767.

FDA (2006). Highlights of Lucentis prescribing information. Available at: http://www.accessdata.fda.gov/drugsatfda_docs/label/2006/125156lbl.pdf.

Ferrara N, Damico L, Shams N, Lowman H & Kim R (2006): Development of ranibizumab, an antivascular endothelial growth factor antigen binding fragment, as therapy for neovascular age-related macular degeneration. Retina **26**: 859–870.

Hassell JB, Lamoureux EL & Keeffe JE (2006): Impact of age related macular degeneration on quality of life. Br J Ophthalmol **90**: 593–596.

Hogan M, Kimura S & Thygeson P (1959): Signs and symptoms of uveitis. I. Anterior uveitis. Am J Ophthalmol **47**: 155–170.

Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2003): Japanese age-related macular degeneration trial: 1-year results of photodynamic therapy with verteporfin in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration. Am J Ophthalmol **136**: 1049–1061.

Japanese Age-Related Macular Degeneration Trial (JAT) Study Group (2008): Photodynamic therapy with verteporfin in Japanese patients with subfoveal choroidal neovascularization secondary to age-related macular degeneration (AMD): results of the Japanese AMD Trial (JAT) extension. Jpn J Ophthalmol **52**: 99–107.

Kawasaki R, Wang JJ, Ji GJ et al. (2008): Prevalence and risk factors for age-related macular degeneration in an adult Japanese population: the Funagata study. Ophthalmology **115**: 1376–1381.

Lowe J, Araujo J, Yang J et al. (2007): Ranibizumab inhibits multiple forms of biologically active vascular endothelial growth factor in vitro and in vivo. Exp Eye Res **85**: 425–430.

Nowak JZ (2006): Age-related macular degeneration (AMD): pathogenesis and therapy. Pharmacol Rep **58**: 353–363.

Oshima Y, Ishibashi T, Murata T, Tahara Y, Kiyohara Y & Kubota T (2001): Prevalence of age related maculopathy in a representative Japanese population: the Hisayama study. Br J Ophthalmol **85**: 1153–1157.

Rosenfeld PJ, Brown DM, Heier JS, Boyer DS, Kaiser PK, Chung CY & Kim RY (2006): Ranibizumab for neovascular age-related macular degeneration. N Engl J Med **355**: 1419–1431.

Received on May 22nd, 2009.
Accepted on November 27th, 2009.

Correspondence:
Masahito Ohji
Department of Ophthalmology
Shiga University of Medical Science
Seta Tsukinowa-cho
Otsu
Shiga 520-2192
Japan
Tel: + 81 77 548 2276
Fax: + 81 77 548 2279
Email: ohji@belle.shiga-med.ac.jp

Macular Pigment Optical Density in Central Serous Chorioretinopathy

Yuzuru Sasamoto,[1] *Fumi Gomi,*[1] *Miki Sawa,*[1] *Motokazu Tsujikawa,*[1] *and Toshimitsu Hamasaki*[2]

Purpose. To evaluate macular pigment optical density (MPOD) in patients with central serous chorioretinopathy (CSC) and in normal subjects.

Methods. MPOD was measured by autofluorescence spectrometry by using a two-wavelength method. Central retinal thickness (CRT) was measured with optical coherence tomography. Statistical analyses were performed to determine factors associated with MPOD.

Results. Ninety-four eyes of 94 normal control subjects, 123 eyes of 70 patients with chronic CSC, and 74 eyes of 41 patients with acute CSC were included. The mean MPOD was 0.548 density unit (DU; 95% confidence interval [CI]; 0.516-0.580) in the control group. Stepwise regression analysis of the control group showed that CRT was associated positively with MPOD ($P = 0.0079$). The mean MPOD was 0.386 DU (95% CI, 0.352-0.420) in the eyes with chronic CSC, 0.443 DU (95% CI, 0.401-0.484) in fellow eyes with chronic CSC, 0.542 DU (95% CI, 0.493-0.590) in affected eyes with acute CSC, and 0.528 DU (95% CI, 0.475-0.582) in fellow eyes with acute CSC. Stepwise regression analysis showed a significant association between eyes with a lower MPOD and affected eyes with chronic CSC ($P = 0.0126$) and fellow eyes with chronic CSC ($P = 0.0023$) and a thinner central retina ($P = 0.0016$).

Conclusions. MPOD may decrease in eyes with chronic CSC and in the fellow eyes. Low MPOD may indicate a risk of chronic CSC, and a decrease in MPOD may be accelerated by thinning of the central retina. (*Invest Ophthalmol Vis Sci.* 2010;51:5219-5225) DOI:10.1167/iovs.09-4881

Macular pigment is composed of three carotenoids (i.e., lutein, zeaxanthin, and *meso*-zeaxanthin). Lutein and zeaxanthin can be obtained only from food, and *meso*-zeaxanthin is synthesized mainly from retinal lutein.[1-6] Macular pigment is distributed primarily in the layer of the fibers of Henle in the fovea and the inner nuclear layer at the parafoveal site.[7]

Macular pigment has light-absorbing properties, with the absorption spectra in the 400- to 540-nm range and the maximum absorption at approximately 460 nm; macular pigment is thought to filter blue light, which is toxic to the photoreceptors.[8-12] In addition, macular pigment itself has an antioxidant effect. It quenches excited triplet states, reacts with singlet oxygen and free radicals, and inhibits peroxidation of long-chain polyunsaturated fatty acids.[6,13-17] Thus, it helps retard some destructive processes in the retina and the retinal pigment epithelium (RPE), which may lead to macular diseases such as age-related macular degeneration (AMD).

Some investigators have tried to determine the amount of macular pigment and elucidate factors affecting macular pigment. Differences in the amount and distribution of macular pigment associated with ethnicity have been reported.[18,19] However, whether macular pigment optical density (MPOD) is affected by aging,[1,15,20-26] sex,[19-21,23,26,27] and smoking[19,20,23,27] is still controversial. In those studies, several clinical methods of measuring MPOD were used, including heterochromatic flickering photometry, motion detection photometry, fundus reflectance spectroscopy, Raman spectrometry, and autofluorescence spectrometry.[28] Among them, autofluorescence spectrometry using a two-wavelength method is independent of the psychophysical methods and is considered to have the highest reproducibility.[22,28-31]

Some research groups have investigated the relationship between AMD and the amount of macular pigment.[1,27,32-35] Obana et al.[32] reported that Japanese patients with AMD have low MPOD. Central serous chorioretinopathy (CSC) is characterized by serous detachment of the neural retina, which also can affect the macula and develop bilaterally.[36,37] Associations with a type A personality, the use of corticosteroids, and pregnancy have been suggested, but the pathogenesis is still unknown.[38-41]

To the best of our knowledge, MPOD in eyes with CSC has not been evaluated. In the present study, we measured MPOD and compared results in Japanese patients with CSC with those in normal subjects. To estimate the factors affecting MPOD in Japanese patients, we performed regression analysis and evaluated the contribution of CSC.

Methods

Study Population

We conducted a cross-sectional observational study at Osaka University Hospital from July 2007 to November 2008. The institutional review board approved the study.

Patients with CSC who met the following criteria in at least one eye were recruited: current or previous episode of serous retinal detachment (SRD) at the macula detected by fundus examination and/or optical coherence tomography (OCT) performed in another institution or our hospital and symptoms of blurred vision, metamorphopsia, micropsia, dyschromatopsia, hypermetropization, or central scotoma in the affected eye. Eyes with other retinal disorders such as rhegmatogenous retinal detachment, choroidal neovascularization, polypoidal choroidal vasculopathy, retinal vein occlusion, macroaneurysms, diabetic retinopathy, and inflammatory eye diseases such as Vogt-Koyanagi-Harada disease were excluded by detailed fundus examina-

From the [1]Department of Ophthalmology and the [2]Department of Biomedical Statistics, Osaka University Medical School, Osaka, Japan.

Supported in part by Bausch & Lomb Japan, Ltd., Tokyo, Japan.

Submitted for publication November 9, 2009; revised March 16, 2010; accepted April 8, 2010.

Disclosure: **Y. Sasamoto**, None; **F. Gomi**, Bausch & Lomb Japan, Ltd. (F); **M. Sawa**, None; **M. Tsujikawa**, None; **T. Hamasaki**, None

Corresponding author: Fumi Gomi, Department of Ophthalmology, Osaka University Medical School, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan; fgomi@ophthal.med.osaka-u.ac.jp.

TABLE 1. Baseline Characteristics

	Control (n = 94)	Chronic CSC (n = 70)		Acute CSC (n = 41)	
		Affected Eyes (n = 63)	Fellow Eyes (n = 47)	Affected Eyes (n = 38)	Fellow Eyes (n = 36)
Eyes meeting criteria	94	76	47	38	36
Sex, n (male/female)	44/50	65/11	39/8	28/10	26/10
Age, y (mean ± SD)	62.8 ± 12.5	55.9 ± 9.6	56.4 ± 10.3	49.6 ± 11.5	50.6 ± 11.7
Smoking, n (yes/no)	34/60	52/23	31/15	25/13	24/11
Missing, n	0	1	1	0	1
RPE damage, n (yes/no)	0/94	33/43	1/46	9/29	0/36
Diffuse RPE atrophy, n (yes/no)	0/94	15/61	0/47	0/38	0/36
SRD, n (yes/no)	0/94	40/36	0/47	27/11	0/36
Size of SRD, disc area (mean ± SD)		1.5 ± 2.4	0	2.9 ± 3.4	0
CRT, μm (mean ± SD)	212.9 ± 20.9	138.2 ± 47.7	207.0 ± 24.4	157.8 ± 37.3	210.5 ± 22.3
Missing, n	51	8	11	4	13
Duration from the onset of latest event, mo (mean ± SD)	0	25.1 ± 36.5	0	2.3 ± 1.5	0

tion and both fluorescein angiography (FA) and indocyanine green angiography (ICGA). Eyes with autofluorescence abnormalities within the area of an annulus with retinal eccentricity of 0.5° also were excluded.

Persistent CSC is sometimes categorized as chronic CSC, but there is no criterion to define it.[42,43] In the present study, we classified eyes with CSC into chronic and acute cases according to the following definition: We defined chronic CSC as that in eyes with episodes of persistent or recurrent SRD during a period of 6 months or more and in which the latest episode of SRD was confirmed within 5 years; acute CSC was defined as that in eyes with current or previous SRD over a period of less than 6 months and that had occurred within 1 year. In all eyes with acute CSC, FA was performed, and the active dye leakage was confirmed. The duration of an SRD was estimated by OCT, fundus photography, or the clinical records at our hospital or the clinics where the patients had been treated.

If both eyes of a patient met the criteria, both eyes were examined as affected eyes, and when one eye did not have apparent abnormalities and no symptoms of CSC, it was examined as a fellow eye.

Subjects without retinal disorders in at least one eye, including healthy volunteers, were recruited for the control group after they agreed to participate in the study. We allowed subjects to participate who had the following retinal diseases in the contralateral eyes: macular hole, idiopathic epiretinal membrane, or rhegmatogenous retinal detachment. The right eye was selected in healthy volunteers in whom both eyes were normal. In all subjects, eyes with high myopia or a substantial cataract were excluded. Subjects with severe renal diseases or those taking supplements containing lutein, zeaxanthin, and/or beta carotene also were excluded. All participants provided informed consent according to the tenets of the Declaration of Helsinki, before MPOD was measured.

Measurement of MPOD

We used a modified retinal angiograph (HRA; Heidelberg Engineering, Heidelberg, Germany) to measure MPOD. The principle of measurement of MPOD with autofluorescence spectrometry with the two-wavelength method has been described.[22,28–30,44,45] Briefly, autofluorescence images were taken of the posterior pole at 488- and 514-nm excitation wavelengths with a band-pass filter at a 530-nm wavelength. Macular pigment absorbs the 488-nm wavelength more than the 514-nm wavelength, and so the subtraction of signals between these two wavelengths creates an MPOD map in the central retina.

All MPOD measurements were performed by two masked orthoptists by using the same testing device and protocol. Before the study, the reliability of MPOD measurements between these two orthoptists was confirmed. Sufficient pupil dilation was obtained by instillation of dilating drops containing 0.5% tropicamide and 2.5% phenylephrine. Subjects sat in front of a table and fixated on an external light source with the fellow eye. If the fellow eye did not have adequate visual acuity (VA) for fixation, the subjects were asked to look straight as much as possible. The modified HRA was aligned with the subject's eye, movies were taken with the 488- and 514-nm excitation wavelengths (scan size, 30°), computed mean autofluorescence images were obtained at each wavelength, and the two images were subtracted to calculate MPOD (expressed as density unit [DU]). The mean MPOD, averaged along the area of an annulus with retinal eccentricity of 0.5° (1° circle at the fovea), was recorded. We examined both eyes if possible.

Eyes were excluded if there was a decrease in the number of effective pixels (≦150/225 pixels), mainly due to poor fixation and failure to detect the fovea with the find-fovea mode. We measured MPOD two or three times at each visit in each eye and then selected the data that varied the least.

Ophthalmic Examinations

The clinical examinations included measurement the best-corrected VA (BCVA) with a Landolt C chart, slit lamp biomicroscopy with a 90-D

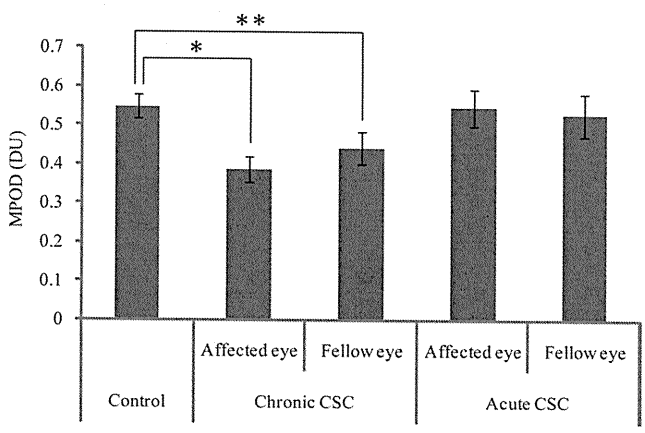


FIGURE 1. MPOD in the control group and the CSC subgroups. The MPOD in the affected eyes and fellow eyes with chronic CSC was significantly lower than in the control eyes (*P < 0.0001 and **P = 0.0005, by the Dunnett test). However, the MPOD in the affected eyes and fellow eyes with acute CSC group did not differ significantly from that in the control eyes.

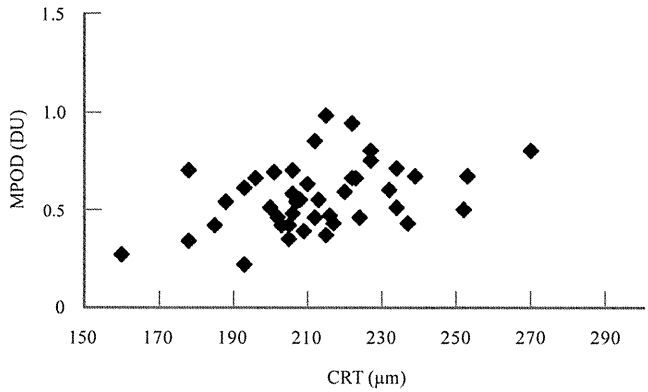


FIGURE 2. The correlation between CRT and MPOD in the control group. The data are plotted for all patients in whom CRT was measured. MPOD rose significantly with increasing CRT ($r = 0.40$; 95% CI, 0.11-0.62).

precorneal lens, digital fundus photography, and OCT (Stratus or Cirrus; Carl Zeiss Meditec, Inc., Dublin, CA), to ascertain the presence of an SRD. When images were obtained with Cirrus OCT, we measured the CRT, defined as the distance between the surface of the inner limiting membrane and the bottom of the sensory retina. Subretinal precipitates were not included in the CRT. FA and ICGA were performed in all eyes with acute CSC and in eyes with persistent SRD in chronic CSC. The presence of RPE damage involving the macula, except for the 1° circle at the fovea, was assessed by examining color fundus photographs, FA, or both. If the size of the areas with RPE damage was 5 disc areas or more, those eyes were defined as having diffuse RPE damage. In eyes with a current SRD, we measured the size of the SRD area from the fundus photograph.

Statistical Analysis

Since the standard deviation of MPOD when including both affected eyes did not differ significantly from data from the unilateral eye of patients with bilateral involvement, all eyes in which MPOD was measured were included in the analysis, even if both eyes of a patient were affected. The baseline characteristics of age, the size of the SRD, CRT, and the time from the onset of the latest event are expressed as the mean ± SD. The mean MPOD and CRT were compared between groups, and the 95% CI was calculated. The two-sided Dunnett *t*-test was used to evaluate the difference in MPOD by group. In the control group, stepwise regression analysis with the Akaike information criterion was performed to determine the covariates that affected MPOD.[46] Pearson's coefficient (r) was applied to the correlation between MPOD and age, the size of the SRD, CRT, and the duration. Stepwise regression analysis was also conducted in all eyes to determine the covariates that affect MPOD in eyes with CSC. To compare the CRT between groups, after we performed stepwise regression analysis to determine the covariates that significantly affect the CRT, analysis of covariance (ANCOVA) and the Dunnett test were performed. $P < 0.05$ was considered significant (SAS software ver. 9.1; SAS Institute, Cary, NC).

RESULTS

A total of 291 eyes of 201 subjects were included (94 eyes of 94 subjects in the control group; 123 eyes of 70 patients in the chronic CSC group; 74 eyes of 41 patients in the acute CSC group). Seventeen patients were affected bilaterally; 13 had bilateral chronic CSC, and 4 had chronic CSC in one eye and acute CSC in the other eye. In the chronic CSC group, 76 eyes were affected and 47 fellow eyes had no apparent abnormalities. In the acute CSC group, 38 eyes were affected and 36 were fellow eyes. The baseline characteristics of all eyes are shown in Table 1.

MPOD in Control and CSC Eyes

The mean MPOD was 0.548 DU (95% CI, 0.516-0.580) in the control group and 0.386 DU (95% CI, 0.352-0.420) in the affected eyes with chronic CSC, 0.443 DU (95% CI, 0.401-0.484) in the fellow eyes with chronic CSC, 0.542 DU (95% CI, 0.493-0.590) in the affected eyes with acute CSC, and 0.528 DU (95% CI, 0.475-0.582) in the fellow eyes with acute CSC. The Dunnett test indicated that MPOD in the affected and fellow eyes with chronic CSC was significantly lower than in the control eyes ($P < 0.0001$ and $P = 0.0005$, respectively; Fig 1). MPOD in the affected and fellow eyes with acute CSC did not differ significantly from that in the control group ($P = 0.9990$ and $P = 0.9306$, respectively).

Factors Affecting MPOD in the Control Group

The overall mean MPOD ± SD was 0.548 ± 0.157 DU ($n = 94$): 0.547 ± 0.141 DU in men ($n = 44$) and 0.549 ± 0.172 DU in women ($n = 50$). The mean ± SD was 0.562 ± 0.149 DU in the subjects who smoked and 0.540 ± 0.163 DU in the nonsmokers. The stepwise regression analysis model, which included sex, age, smoking, and CRT as explanatory covariates and MPOD as a response, showed that CRT was the most important covariate associated with MPOD ($P = 0.0079$). The other covariates were not associated significantly with MPOD. Although the mean ± SD baseline ages in the subgroups were the same (Table 1), stepwise regression analysis did not identify

TABLE 2. Relationship between MPOD and Covariates in the Affected Eyes with Chronic CSC

	MPOD		*P*
Male/female	0.387 (0.350-0.424) ($n = 65$)	0.380 (0.283-0.477) ($n = 11$)	0.8849
Smoking, yes/no	0.385 (0.343-0.427) ($n = 52$)	0.388 (0.322-0.455) ($n = 23$)	0.9313
RPE damage, yes/no	0.364 (0.314-0.413) ($n = 33$)	0.403 (0.356-0.451) ($n = 43$)	0.2515
Diffuse RPE damage, yes/no	0.385 (0.308-0.462) ($n = 15$)	0.386 (0.348-0.425) ($n = 61$)	0.9681
SRD, yes/no	0.380 (0.333-0.426) ($n = 40$)	0.393 (0.341-0.445) ($n = 36$)	0.6992

	Correlation Coefficient	*P*
Age ($n = 76$)	−0.14	0.2366
Size of SRD ($n = 40$)	0.05	0.7583
CRT ($n = 68$)	0.28	0.0210
Duration ($n = 76$)	−0.34	0.0026

MPOD data are expressed as the mean DU (95% CI).

TABLE 3. Relationship between the MPOD Level and Covariates in the Affected Eyes with Acute CSC

	MPOD		P
Male/female	0.553 (0.499-0.607) (n = 28)	0.510 (0.390-0.630) (n = 10)	0.4343
Smoking, yes/no	0.525 (0.463-0.587) (n = 25)	0.573 (0.489-0.657) (n = 13)	0.3458
RPE damage, yes/no	0.587 (0.469-0.704) (n = 9)	0.528 (0.473-0.582) (n = 29)	0.2965
Diffuse RPE damage, yes/no	— (n = 0)	0.542 (0.493-0.590) (n = 38)	—
SRD, yes/no	0.530 (0.478-0.582) (n = 27)	0.579 (0.466-0.693) (n = 12)	0.3389
	Correlation Coefficient		**P**
Age (n = 38)	−0.05		0.7617
Size of SRD (n = 27)	0.30		0.1320
CRT (n = 35)	0.16		0.3575
Duration (n = 38)	0.21		0.2005

MPOD data are expressed as DU (95% CI).

age as an important covariate. Figure 2 shows the correlation between CRT and MPOD. MPOD rose significantly with increases in CRT (r = 0.40; 95% CI, 0.11-0.62).

Factors Affecting MPOD in All Eyes

The relationship between MPOD in the affected eyes with acute and chronic CSC and the covariates (i.e., age, sex, smoking, RPE damage, the presence or absence of an SRD, the size of the SRD, and the time from the onset of the latest event), are shown in Tables 2 and 3, respectively. MPOD correlated significantly with CRT (r = 0.28, P = 0.0210) and the time from the onset of the latest event (r = −0.34, P = 0.0026) in the affected eyes with chronic CSC. The other covariates did not correlate significantly with MPOD. To determine the major factors associated with MPOD, we performed stepwise regression analyses in all eyes, including the control group and the CSC subgroups (Table 4). RPE damage, SRD, and the CSC subgroups were included as explanatory covariates along with sex, age, smoking, and CRT. Because the duration was directly associated with the definition of chronic or acute and correlated strongly with CRT (r = −0.31, P = 0.0017), it was not included in this analysis. Stepwise regression analysis showed that CRT and the affected eyes and fellow eyes with chronic CSC were the important covariates associated with MPOD (P = 0.0016, P = 0.0126, and P = 0.0023, respectively). The smokers also tended to have a lower MPOD than did the nonsmokers (P = 0.1320). The other covariates had little effect on MPOD.

The results of stepwise regression analysis showed that CRT and CSC affected MPOD independently. To confirm the relationship between CRT and CSC, we used stepwise regression analysis and ANCOVA to analyze the factors that affected CRT.

CRT in the CSC eyes ranged from 44 to 289 μm (mean ± SD, 168.0 ± 49.3). Because stepwise regression analysis identified RPE damage, SRD, and the CSC subgroups as significant covariates associated with CRT among the sex, age, smoking, RPE damage, SRD, and CSC subgroups, we performed ANCOVA and the Dunnett test to compare CRT among the CSC subgroups. The adjusted mean CRT was 181.9 μm (95% CI, 168.3-195.4) in the control group, 138.4 μm (95% CI, 130.5-146.2) in the affected eyes with chronic CSC, 176.7 μm (95% CI, 162.4-190.9) in the fellow eyes with chronic CSC, 159.2 μm (95% CI, 147.2-171.3) in the affected eyes with acute CSC, and 179.2 μm (95% CI, 162.7-195.8) in the fellow eyes with acute CSC (Fig. 3). The Dunnett test showed that the central retinas in the affected eyes with chronic and acute CSC were thinner than those in the control group (P < 0.0001 and P = 0.0487, respectively). However, no significant differences were found in the CRT in the fellow eyes with chronic and acute CSC (P = 0.9019 and P = 0.9945, respectively).

DISCUSSION

We used autofluorescence spectrometry to evaluate MPOD in a control group and in two groups of patients with CSC. Among the several methods of estimating MPOD, this one is considered to have the highest reproducibility.[22,28-31] Autofluorescence spectrometry has shown ethnic differences in the level and distribution of macular pigments[18,19]; however, only one study reported MPODs in a Japanese population in which resonance Raman spectroscopy was used.[32] Therefore, in the present study, we were the first to examine MPOD in both normal and CSC eyes in the Japanese, by using autofluorescence spectrometry.

In the study, MPOD at an eccentricity of 0.5° was 0.548 ± 0.157 DU in normal subjects. Previously, Liew et al.[47] and Trieschmann et al.[48] reported that the mean MPOD ± SD in normal subjects was 0.41 ± 0.15 and 0.50 ± 0.19 DU, respectively. Compared with those reports, the present study showed that the SD of MPOD was almost the same and the mean MPOD was significantly higher. Wolf-Schnurrbusch et al.[18] reported that individuals of African descent had significantly higher MPOD than white non-Hispanics. The current data also sug-

TABLE 4. Selected Covariates in all Eyes Affecting Macular Pigment Optical Density

Selected Variable	Regression Coefficient (95% CI)	t	P
Constant	0.344 (0.190 to 0.498)	5.03	<0.0001
Smoking, yes	−0.033 (−0.082 to 0.016)	−1.51	0.1320
CRT	0.001 (0.000 to 0.002)	3.21	0.0016
Chronic CSC, affected eye	−0.093 (−0.176 to −0.010)	−2.52	0.0126
Chronic CSC, fellow eye	−0.105 (−0.181 to −0.028)	−3.08	0.0023
Acute CSC, affected eye	0.055 (−0.030 to 0.141)	1.47	0.1444
Acute CSC, fellow eye	−0.048 (−0.136 to 0.039)	−1.25	0.2145

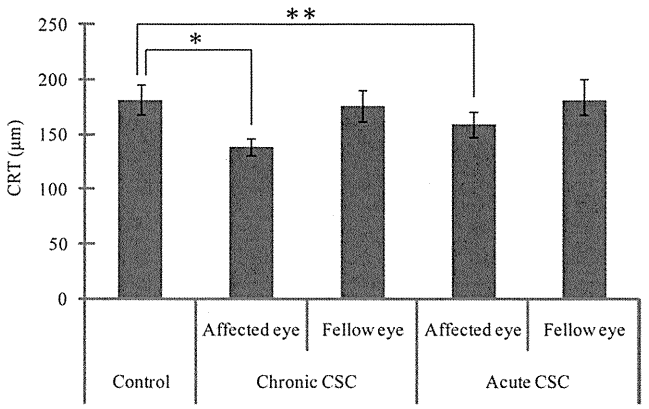


FIGURE 3. CRT in the control group and the CSC subgroups. After adjustment for age, the CRT in the affected eyes with chronic and acute CSC was significantly less than in the control group (*$P < 0.0001$ and **$P = 0.0487$, by the Dunnett test). However, the CRT in the fellow eyes with chronic and acute CSC did not differ significantly from the control group.

gested that MPOD in Asian individuals may be higher than in Caucasian people.

The present study showed that CRT was moderately positively correlated with MPOD in normal subjects, and regression analysis also showed a significant association between MPOD and CRT. Macular pigment is distributed in the layer of the fibers of Henle and the inner retinal layer, which suggests that MPOD may change along with the morphologic retinal changes—that is, MPOD per unit volume of retina is constant to some extent. Liew et al.[47] also showed a significant but modest relationship between CRT and MPOD. Nolan et al.[49] recently reported that the relationship between the CRT and MPOD was positive and significant in non-Caucasians but not in Caucasian subjects. Thus, in normal subjects, CRT may be positively associated with MPOD, but sex, age, and smoking may not be associated significantly with MPOD in a Japanese population.

We then evaluated the association between MPOD and CSC. The mechanisms of the development of CSC are still unknown, but there may be some differences in developing disease between acute and chronic CSC. The VA prognosis in acute CSC is usually good; however, it is often worse in chronic CSC because of persistent subretinal exudation and extensive atrophy of the retina and RPE. In addition, more eyes with chronic CSC develop SRD bilaterally and have repeated episodes.[37] The mean MPOD in all eyes with CSC was 0.456 DU (95% CI, 0.433-0.478), which differed significantly from the control eyes ($P < 0.0001$). Among the characteristics in affected eyes, the CRT correlated positively with MPOD in the affected eyes with chronic CSC, as seen in normal subjects. The time from the onset of the latest event, which may correlate inversely with the CRT, correlated negatively ($r = -0.31$, $P = 0.0017$) with MPOD in the affected eyes with chronic CSC.

When it was compared between the control group and the CSC subgroups, MPOD in the affected and fellow eyes with chronic CSC was significantly lower than in the control eyes (Fig 1). To confirm this in detail, stepwise regression analysis was performed that included all the examined eyes and showed that CRT and affected and fellow eyes with chronic CSC were the factors that were significantly associated with MPOD. Sex, age, RPE damage, and SRD in the affected eyes were not associated significantly with MPOD. The central retina in eyes with chronic CSC is thin and correlates with disease duration based on OCT studies.[50,51] The present study also showed that the central retina in eyes with chronic and acute CSC was significantly thinner than in normal subjects. Therefore, a thinner central retina may result in a low MPOD in the affected eyes with chronic CSC for the same reason as in normal eyes.

The duration of the SRD also was considered to be associated with lower MPOD, independent of the morphologic retinal changes in eyes with chronic CSC. The long-term persistence of subretinal fluid may disrupt the macular pigment supply from the RPE-choroid complex[52] and cause a shortage in the retina. These possibilities suggest that the lower MPOD in CSC eyes is the result of the disease.

However, the results of regression analysis showed that the affected and fellow eyes with chronic CSC had significantly lower MPOD independent of CRT. This result suggests that eyes with low MPOD are likely to develop chronic CSC, but not as a result of persistent disease. This finding means that eyes with chronic CSC without thinning of the central retina had low MPOD, and the thinner central retina resulted in much lower MPOD. This hypothesis is supported by the finding of lower MPOD in fellow eyes with chronic CSC, although the CRT was maintained.

The possibility should be considered that the low MPOD in eyes with chronic CSC results from methodologic artifacts of autofluorescence spectrometry. The increased fundus autofluorescence (FAF) in eyes with CSC is well known.[53] Eyes with autofluorescence abnormalities within the area of an annulus with retinal eccentricity of 0.5° were excluded from the present study, but if unknown substances reduce the absorption of FAF in the macular pigment at 488 nm or increase the absorption at 514 nm, autofluorescence spectrometry may measure lower MPOD.

In conclusion, low MPOD may be a risk factor for the development of chronic CSC, and a decrease in MPOD may be accelerated by a thinning central retina. A limitation of the present study was that MPOD in most eyes was measured once, not repeatedly, along with disease progression or persistence. We also could not exclude the possibility that a few asymptomatic eyes with CSC were included among the fellow eyes, despite detailed ophthalmic examinations including OCT. In addition, MPOD may vary interindividually, even when measured by autofluorescence spectrometry,[30,47,48] probably because of difficulties in the standardization of individually different intensities and distributions of fundus autofluorescence. However, because we measured MPOD in a relatively large number of eyes, we believe that our data are meaningful. The exact relationship between low MPOD and development of chronic CSC is unknown; however, our data suggest the possibility that the supplementation of macular pigment suppresses the development or progression of chronic CSC, which causes substantial visual deterioration. Further studies are needed.

Acknowledgments

The authors are grateful for the immeasurable contribution of the late Yasuo Tano to this study.

References

1. Beatty S, Murray IJ, Henson DB, Carden D, Koh H, Boulton ME. Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. *Invest Ophthalmol Vis Sci.* 2001;42:439-446.
2. Berendschot TT, Goldbohm RA, Klopping WA, van de Kraats J, van Norel J, van Norren D. Influence of lutein supplementation on macular pigment, assessed with two objective techniques. *Invest Ophthalmol Vis Sci.* 2000;41:3322-3326.

3. Bone RA, Landrum JT, Cains A. Optical density spectra of the macular pigment in vivo and in vitro. *Vision Res.* 1992;32:105-110.
4. Bone RA, Landrum JT, Fernandez L, Tarsis SL. Analysis of the macular pigment by HPLC: retinal distribution and age study. *Invest Ophthalmol Vis Sci.* 1988;29:843-849.
5. Neuringer M, Sandstrom MM, Johnson EJ, Snodderly DM. Nutritional manipulation of primate retinas, I: effects of lutein or zeaxanthin supplements on serum and macular pigment in xanthophyll-free rhesus monkeys. *Invest Ophthalmol Vis Sci.* 2004;45:3234-3243.
6. Sommerburg OG, Siems WG, Hurst JS, Lewis JW, Kliger DS, van Kuijk FJ. Lutein and zeaxanthin are associated with photoreceptors in the human retina. *Curr Eye Res.* 1999;19:491-495.
7. Trieschmann M, van Kuijk FJ, Alexander R, et al. Macular pigment in the human retina: histological evaluation of localization and distribution. *Eye.* 2008;22:132-137.
8. Kirschfeld K. Carotenoid pigments: their possible role in protecting against photooxidation in eyes and photoreceptor cells. *Proc R Soc Lond B Biol Sci.* 1982;216:71-85.
9. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. *Arch Biochem Biophys.* 2001;385:28-40.
10. Rapp LM, Maple SS, Choi JH. Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol Vis Sci.* 2000;41:1200-1209.
11. Snodderly DM. Evidence for protection against age-related macular degeneration by carotenoids and antioxidant vitamins. *Am J Clin Nutr.* 1995;62:1448S-1461S.
12. Snodderly DM, Brown PK, Delori FC, Auran JD. The macular pigment. I. Absorbance spectra, localization, and discrimination from other yellow pigments in primate retinas. *Invest Ophthalmol Vis Sci.* 1984;25:660-673.
13. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol.* 1999;83:867-877.
14. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol.* 2000;45:115-134.
15. Gellermann W, Ermakov IV, Ermakova MR, McClane RW, Zhao DY, Bernstein PS. In vivo resonant Raman measurement of macular carotenoid pigments in the young and the aging human retina. *J Opt Soc Am A Opt Image Sci Vis.* 2002;19:1172-1186.
16. Landrum JT, Bone RA, Kilburn MD. The macular pigment: a possible role in protection from age-related macular degeneration. *Adv Pharmacol.* 1997;38:537-556.
17. Snodderly DM, Russett MD, Land RI, Krinsky NI. Plasma carotenoids of monkeys (Macaca fascicularis and Saimiri sciureus) fed a nonpurified diet. *J Nutr.* 1990;120:1663-1671.
18. Wolf-Schnurrbusch UE, Roosli N, Weyermann E, Heldner MR, Hohne K, Wolf S. Ethnic differences in macular pigment density and distribution. *Invest Ophthalmol Vis Sci.* 2007;48:3783-3787.
19. Iannaccone A, Mura M, Gallaher KT, et al. Macular pigment optical density in the elderly: findings in a large biracial Mid-south population sample. *Invest Ophthalmol Vis Sci.* 2007;48:1458-1465.
20. Hammond BR, Jr., Caruso-Avery M. Macular pigment optical density in a Southwestern sample. *Invest Ophthalmol Vis Sci.* 2000;41:1492-1497.
21. Lam RF, Rao SK, Fan DS, Lau FT, Lam DS. Macular pigment optical density in a Chinese sample. *Curr Eye Res.* 2005;30:799-805.
22. Liew SH, Gilbert CE, Spector TD, et al. Heritability of macular pigment: a twin study. *Invest Ophthalmol Vis Sci.* 2005;46:4430-4436.
23. Mellerio J, Ahmadi-Lari S, van Kuijk F, Pauleikhoff D, Bird A, Marshall J. A portable instrument for measuring macular pigment with central fixation. *Curr Eye Res.* 2002;25:37-47.
24. Nolan JM, Stack J, O'Donavan O, Loane E, Beatty S. Risk factors for age-related maculopathy are associated with a relative lack of macular pigment. *Exp Eye Res.* 2007;84:61-74.
25. Werner JS, Bieber ML, Schefrin BE. Senescence of foveal and parafoveal cone sensitivities and their relations to macular pigment density. *J Opt Soc Am A Opt Image Sci Vis.* 2000;17:1918-1932.
26. Wustemeyer H, Moessner A, Jahn C, Wolf S. Macular pigment density in healthy subjects quantified with a modified confocal scanning laser ophthalmoscope. *Graefes Arch Clin Exp Ophthalmol.* 2003;241:647-651.
27. Jahn C, Wustemeyer H, Brinkmann C, Trautmann S, Mossner A, Wolf S. Macular pigment density in age-related maculopathy. *Graefes Arch Clin Exp Ophthalmol.* 2005;243:222-227.
28. Leung IY. Macular pigment: new clinical methods of detection and the role of carotenoids in age-related macular degeneration. *Optometry.* 2008;79:266-272.
29. Delori FC. Autofluorescence method to measure macular pigment optical densities fluorometry and autofluorescence imaging. *Arch Biochem Biophys.* 2004;430:156-162.
30. Trieschmann M, Heimes B, Hense HW, Pauleikhoff D. Macular pigment optical density measurement in autofluorescence imaging: comparison of one- and two-wavelength methods. *Graefes Arch Clin Exp Ophthalmol.* 2006;244:1565-1574.
31. Wolf S. Macular pigment measurements: which method should we use? *Graefes Arch Clin Exp Ophthalmol.* 2006;244:1562-1564.
32. Obana A, Hiramitsu T, Gohto Y, et al. Macular carotenoid levels of normal subjects and age-related maculopathy patients in a Japanese population. *Ophthalmology.* 2008;115:147-157.
33. Berendschot TT, Willemse-Assink JJ, Bastiaanse M, de Jong PT, van Norren D. Macular pigment and melanin in age-related maculopathy in a general population. *Invest Ophthalmol Vis Sci.* 2002;43:1928-1932.
34. LaRowe TL, Mares JA, Snodderly DM, Klein ML, Wooten BR, Chappell R. Macular pigment density and age-related maculopathy in the Carotenoids in Age-Related Eye Disease Study: an ancillary study of the women's health initiative. *Ophthalmology.* 2008;115:876-883.e871.
35. Trieschmann M, Spital G, Lommatzsch A, et al. Macular pigment: quantitative analysis on autofluorescence images. *Graefes Arch Clin Exp Ophthalmol.* 2003;241:1006-1012.
36. Green RP Jr, Carlson DW, Dieckert JP, Tredici TJ. Central serous chorioretinopathy in U.S. Air Force aviators: a review. *Aviat Space Environ Med.* 1988;59:1170-1175.
37. Torron C, Melcon B, Ferrer E, Ruiz O, Olivan JM, Honrubia FM. Central serous choroidopathy: long term study (in Spanish). *Arch Soc Esp Oftalmol.* 2000;75:103-108.
38. Yannuzzi LA. Type A behavior and central serous chorioretinopathy. *Trans Am Ophthalmol Soc.* 1986;84:799-845.
39. Polak BC, Baarsma GS, Snyers B. Diffuse retinal pigment epitheliopathy complicating systemic corticosteroid treatment. *Br J Ophthalmol.* 1995;79:922-925.
40. Tittl MK, Spaide RF, Wong D, et al. Systemic findings associated with central serous chorioretinopathy. *Am J Ophthalmol.* 1999;128:63-68.
41. Haimovici R, Koh S, Gagnon DR, Lehrfeld T, Wellik S. Risk factors for central serous chorioretinopathy: a case-control study. *Ophthalmology.* 2004;111:244-249.
42. Framme C, Walter A, Gabler B, Roider J, Sachs HG, Gabel VP. Fundus autofluorescence in acute and chronic-recurrent central serous chorioretinopathy. *Acta Ophthalmol Scand.* 2005;83:161-167.
43. Hussain N, Khanna R, Hussain A, Das T. Transpupillary thermotherapy for chronic central serous chorioretinopathy. *Graefes Arch Clin Exp Ophthalmol.* 2006;244:1045-1051.
44. Delori FC, Goger DG, Hammond BR, Snodderly DM, Burns SA. Macular pigment density measured by autofluorescence spectrometry: comparison with reflectometry and heterochromatic flicker photometry. *J Opt Soc Am A Opt Image Sci Vis.* 2001;18:1212-1230.
45. Wustemeyer H, Jahn C, Nestler A, Barth T, Wolf S. A new instrument for the quantification of macular pigment density: first results in patients with AMD and healthy subjects. *Graefes Arch Clin Exp Ophthalmol.* 2002;240:666-671.