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MEDICAL OPHTHALMOLOGY

Effect of 1-year lutein supplementation on macular pigment optical density and visual function

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Abstract
Background Although it is known that antioxidants including lutein can affect macular pigment optical density (MPOD) and visual function, we still have much to learn about their effect. Our aim was to assess the 1-year changes in MPOD and visual function in response to supplementation containing lutein.
Methods We prospectively measured the MPOD level of those who received a supplement containing 6 mg of lutein daily for 1 year. MPOD level was measured every 3 months by using autofluorescence spectrometry with the two-wavelength method. Other examinations, including contrast sensitivity and retinal sensitivity were also measured every 3 or 6 months. Stepwise regression analysis was performed to determine the factors that correlated with the changes observed in those examinations.
Results Forty-three eyes of 43 Japanese subjects, including five normal eyes, five fellow eyes with central serous chorioretinopathy (CSC), and 33 fellow eyes with age-related macular degeneration (AMD) were enrolled. The higher baseline MPOD level was correlated with the eye with a clear intraocular lens (IOL). Although no time-dependent changes in the MPOD level were obtained in any area, subjects without cardiovascular diseases showed higher increase in the MPOD level. We observed significant increases in the contrast sensitivity at 1 year (p=0.0124) and in the retinal sensitivity at 6 months (p<0.0001) and 1 year (p<0.0001). Stepwise regression analysis showed that nonsmokers had increased contrast sensitivity (p=0.0173), and the fellow eye of those with CSC had less of an increase in retinal sensitivity (p=0.0491).
Conclusions Daily supplementation with 6 mg of lutein did not affect the MPOD level for 1 year, suggesting that 6 mg of lutein may be insufficient to increase the MPOD level. However, supplementation seems to improve visual functions such as contrast sensitivity and retinal sensitivity.

Keywords Macular pigment · Fundus autofluorescence · Lutein · Contrast sensitivity · Microperimetry

Introduction

Macular pigment, which is comprised of three carotenoids, i.e., lutein, zeaxanthin, and meso-zeaxanthin [1–3], has light-absorbing properties in the 400- to 540-nm range, with maximum absorption at about 460 nm [3–5]. In addition, the macular pigment itself has an antioxidative effect [3, 6–9]. Thus, macular pigment may help retard some destructive processes in the retina and the retinal pigment epithelium, which can cause macular diseases such as age-related maculopathy, age-related macular degeneration (AMD), and possibly central serous chorioretinopathy (CSC) [10–15].

Several studies have investigated the relationship between dietary supplementation with lutein and zeaxanthin and macular pigment optical density (MPOD). It is controversial whether supplementation with these carote-

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noids and/or other antioxidants increases the MPOD level and consequently prevents the development of AMD [16–28]. The role of macular pigment and vitamins in visual function, such as visual acuity (VA) and contrast sensitivity, has also been discussed [28–36]. Some studies have reported that the supplemental antioxidants had a positive effect on visual function, but other studies did not.

In the current study, we investigated the MPOD level in response to supplementation with lutein and other antioxidants and minerals in 43 eyes of 43 Japanese subjects without abnormal fundus autofluorescence (FAF) at the fovea. We also estimated the effect of this supplementation on visual functions, including VA, contrast sensitivity, and retinal sensitivity. To the best of our knowledge, this is the first study to report a relationship between supplementation with carotenoids and time-dependent changes in the MPOD levels and visual function in a Japanese population.

Materials and methods

Study population

We conducted a prospective interventional study at Osaka University Hospital from January 2008 to October 2009. The institutional review board approved this study.

We calculated the power (number of eyes) needed for this study, the primary outcome of which was the changes in the MPOD level averaged along the area of an annulus with a retinal eccentricity of 0.5 degree. Considering the normal data that we previously reported, we found that 39 eyes were needed to detect a change in the MPOD optical density of 0.10 density units (DUs) [15]. Because we expected dropout during the follow-up period, we enrolled 43 consecutive Japanese subjects over 40 years old who had never taken supplementation previously. Written informed consent was obtained from all subjects enrolled.

Eyes with less than 0.05 of logMAR score and without apparent retinal disorders and abnormal FAF including the fovea were recruited. All subjects had undergone the detailed fundus examination, optical coherence tomography, and fundus autofluorescence (FAF), and if any macular disorders were suspected, fluorescein and indocyanine green angiography was performed and excluded the subjects with bilateral disease. When one eye had retinal disorders such as CSC and AMD and fellow eyes confirmed to have no apparent abnormalities including FAF examination at the fovea, we selected the fellow eye as the target of our study. Eyes with dry AMD were not included. If both eyes had no ocular disorders, we selected the right eye.

Subjects took daily supplements of 6 mg of lutein and other vitamins and minerals (Ocuvite plus Lutein, Bausch & Lomb Japan, Tokyo, Japan) for 1 year and were examined every 3 months. The ingredients in Ocuvite plus Lutein are shown in Table 1. All participants provided informed consent at the beginning of the supplementation according to the tenets of the Declaration of Helsinki.

Measurement of the MPOD level

We measured the MPOD level in all eyes using the modified Heidelberg Retina Angiograph (HRA, Heidelberg Engineering, Dossenheim, Germany) every 3 months (0, 3, 6, 9, and 12 months). Autofluorescence spectrometry with the two-wavelength method was the principle measurement of the MPOD [37–39]. Two masked orthoptists who used the same testing device and protocol performed all measurements. Before the study, the reliability of the measurements between the two orthoptists was confirmed as reported previously [15].

Before the measurements, sufficient pupil dilation was obtained with instillation of dilating drops containing 0.5% tropicamide and 2.5% phenylephrine. The subjects sat at a table and fixated on an external light source with the fellow eye. If the fellow eye did not have adequate VA for fixation, the subjects were asked to look straight as much as possible. The modified HRA was aligned with the subject's eye, and movies were taken with the 488- and 514-nm excitation wavelengths (scan size, 30 degrees); computed mean autofluorescence images were obtained at each wavelength, and the two images were subtracted to calculate the MPOD level expressed in DUs. In accordance with other studies using this method, we chose the mean MPOD level averaged along the area of an annulus with a retinal eccentricity of 0.5 degree (MPOD level on 0.5 degree) as a primary outcome. We also calculated the MPOD level averaged within the area of an annulus with a retinal eccentricity of 0.5 degree (MPOD level within 0.5 degree) and 1.0 degree (MPOD level within 1.0 degree). We measured the MPOD two or three times in each eye during

Table 1 Ingredients in Ocuvite plus Lutein

Lutein	6 mg
Beta-carotene (provitamin A)	1200 μg
Vitamin C	300 mg
Vitamin E	60 mg
Vitamin B_2	3 mg
Niacin	12 mg
Zinc	9 mg
Selenium	45 μg
Copper	0.6 mg
Manganese	1.5 mg

each visit and then selected the data with the best quality image [37].

Ophthalmic examinations

To determine the effect of supplementation on visual function, we obtained the best spectacle corrected VA (BCVA) levels using Landolt C charts that then were converted to the logarithm of the minimal angle of resolution (logMAR) score, the low-contrast VA measured using the CSV-1000 LanC10% (Vector Vision Co., Greenville, OH), the contrast sensitivity using the CSV-1000E (Vector Vision Co.), and the mean retinal sensitivity measured by the MP-1 Microperimeter (Nidek Technologies, Padova, Italy).

The BCVA was measured at the beginning and after 1 year of supplementation. Low-contrast VA testing was measured at 0, 3, 6, 9, and 12 months to identify minute changes in visual function [40]. The CSV-1000LanC10% is based on the Early Treatment Diabetic Retinopathy Study (ETDRS) chart (The Lighthouse, New York). This chart displays five letters per line at 10% low contrast. The low-contrast VA was scored by the number of letters identified correctly with full spectacle correction.

Contrast sensitivity usually is measured at several different spatial frequencies. We tested the contrast sensitivity at 3, 6, 12, and 18 cycles per degree (cpd). The contrast level of the last correct response for each cpd was recorded as the contrast threshold in logarithmic value under full spectacle correction. We then calculated the area under the log contrast sensitivity function (AULCSF) as representative of the contrast sensitivity data according to the method of Applegate et al. [41]. The log contrast sensitivity versus log spatial frequency data were fitted to a third-order polynomial curve. The fitted function was integrated between the fixed limits of log spatial frequencies of 0.48 (corresponding to 3 cpd) and 1.26 (18 cpd), and the resulting value was defined as the AULCSF.

Microperimetry was performed at 0, 6, and 12 months [42, 43]. The retinal sensitivity threshold was measured using Goldmann III stimuli (circle with a white background) projected on a white background with background illumination of 1.27 cd/m^2 and stimulus presentation time of 200 ms. We tested a radial grid of five stimuli within the area of an annulus with a retinal eccentricity of 0.5 degree and that of 45 stimuli within the area of an annulus with a retinal eccentricity of 6.0 degrees.

Statistical analysis

The baseline data are expressed as the mean ± standard deviation (SD).

Stepwise regression analysis using the Akaike information criteria was performed to determine the covariates that affected the MPOD level of each area at baseline, i.e., gender, age, smoking, lens status, cardiovascular diseases (stroke, angina, and myocardial infarction), disease in the fellow eye, low-contrast VA, AULCSF, and retinal sensitivity within 0.5 degree [44]. We did not select the logMAR VA as a covariate because low-contrast VA is more sensitive than logMAR VA and they are moderately correlated with each other (r=−0.63, p<0.0001). Similarly, we did not select retinal sensitivity within 6.0 degrees as a covariate because the MPOD level was measured within 1.0 degree and the retinal sensitivity within 0.5 degree and 6.0 degrees are strongly correlated with each other (r=0.86, p<0.0001). Stepwise regression analysis was conducted to detect the covariates that correlated with the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 and 6.0 degrees at baseline, including gender, age, smoking, lens status, cardiovascular diseases and disease in the fellow eye plus low-contrast VA at baseline when evaluating the AULCSF and the retinal sensitivity.

To estimate the differences compared with baseline at each time point (3, 6, 9, and 12 months) within the variables, we calculated the 95% CI and performed a paired t test and the significance level was adjusted by Bonferroni correction. That is, because we considered $p \leqq 0.05$ significant when comparing just one time point with baseline, $p \leqq 0.025$ and $p \leqq 0.0125$, respectively, were considered significant when comparing two and four time points with baseline. To determine the variables that affected the 1-year change in the MPOD level, stepwise regression analysis was performed, where gender, age, smoking, lens status, cardiovascular diseases, disease in the fellow eye, MPOD on the 0.5 degree, the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 degree at baseline were included as covariates. Stepwise regression analysis was also conducted to detect the covariates that correlated with the changes in the low-contrast VA, the AULCSF, and the retinal sensitivity within 0.5 and 6.0 degrees, including gender, age, smoking, lens status, cardiovascular diseases, disease in the fellow eye, MPOD on the 0.5 degree at baseline, and the low-contrast VA at baseline plus the baseline value of the respective factors.

JMP software version 8.0 (SAS Institute Inc., Cary, NC) was used for statistical analysis.

Results

Baseline characteristics

A total of 43 eyes of 43 subjects (26 men, 17 women; mean age ± SD, 64.5±9.1 years) were included. Twenty-seven

subjects were smokers and 16 subjects were nonsmokers. Six subjects had had a past history of cardiovascular diseases (stroke, angina, and myocardial infarction). Three eyes had undergone cataract surgery with implantation of clear intraocular lens (IOL) before the study. Five eyes were those of healthy volunteers, five eyes were fellow eyes of those with CSC, and 33 eyes were fellow eyes of those with AMD (18 eyes were polypoidal choroidal vasculopathy, 12 eyes were exudative AMD, two eyes were age-related maculopathy and one eye was retinal angiomatous proliferation). None of the subjects had an unbalanced diet. The subjects had spherical equivalent between −3.88 diopter (D) and 4.63 D (mean ± SD was 0.11±1.88 D) and cylinder magnitude up to 3.50 D. Although 16 eyes had minimal retinal pigment epithelium damage within 6.0 degrees, foveal regions in those eyes were not affected.

Accurate MPOD levels were obtained from all subjects. The mean ± SD MPOD level measured by autofluorescence spectrometry was 0.480±0.136 DU (on 0.5 degree), 0.493±0.138 DU (within 0.5 degree), and 0.474±0.134 DU (within 1.0 degree). The mean±SD logMAR BCVA was −0.078±0.077 (the mean Snellen equivalent; 24/20), and the low-contrast VA was 77.7±6.3 letters. The mean±SD AULCSF was 1.36±0.14, and the retinal sensitivity was 14.8±3.2 dB (within 0.5 degree) and 15.3±2.4 dB (within 6.0 degrees).

Stepwise regression analysis showed that the higher baseline MPOD levels on 0.5 degree and within 0.5 degree were correlated with the eye with clear IOL (T=2.65, p=0.0114; and T=2.53, p=0.0152, respectively). The low-contrast VA and the retinal sensitivity within 0.5 degree and 6.0 degrees were correlated negatively with age (T=−4.07, p=0.0002; T=−2.37, p=0.0225; and T=−3.75, p=0.0006, respectively). The AULCSF showed no significant correlation with any covariates.

Time-dependent changes in MPOD

During follow-up, all subjects took daily supplementation containing 6 mg of lutein. No enrolled eyes developed any ocular disorders during the follow-up period.

The time-dependent changes in the MPOD level on 0.5 degree are shown in Fig. 1a. The MPOD levels were 0.480 DU (95% CI, 0.438, 0.521) at baseline, 0.480 DU (95% CI, 0.439, 0.521) at 3 months, 0.485 DU (95% CI, 0.443, 0.527) at 6 months, 0.484 DU (95% CI, 0.442, 0.527) at 9 months, and 0.488 DU (95% CI, 0.445, 0.532) at 12 months. The MPOD differences between the beginning of supplementation (baseline) and each follow-up time point (3, 6, 9, and 12 months) were calculated by paired t test; the respective p values were as p=0.9558, p=0.4386, p=0.5183, and p=0.2610. There were no significant changes in the MPOD level at any time point during the follow-up period when compared to a significance level of 0.0125 adjusted by Bonferroni correction. We also measured the mean MPOD level within 0.5 and 1.0 degree (Fig. 1b). There were also no significant time-dependent changes in the MPOD levels in those areas. Stepwise regression analysis showed that the 1-year increase in the MPOD levels on 0.5 degree and within 0.5 degree were correlated with subjects with no cardiovascular diseases (T=2.20, p=0.0338; and T=2.42, p=0.0201, respectively), and 1 year decrease in the MPOD level within 1.0 degree was correlated with phakic eye (T=−3.96, p=0.0003).

Time-dependent changes in visual function

The mean baseline logMAR was −0.078 (95% CI, -0.102, -0.055) (the Snellen equivalent; 24/20), which did not differ significantly from the mean logMAR at 12 months, -0.096 (95% CI, -0.122, -0.069) (the Snellen equivalent; 25/20) (p=0.1658, paired t-test).

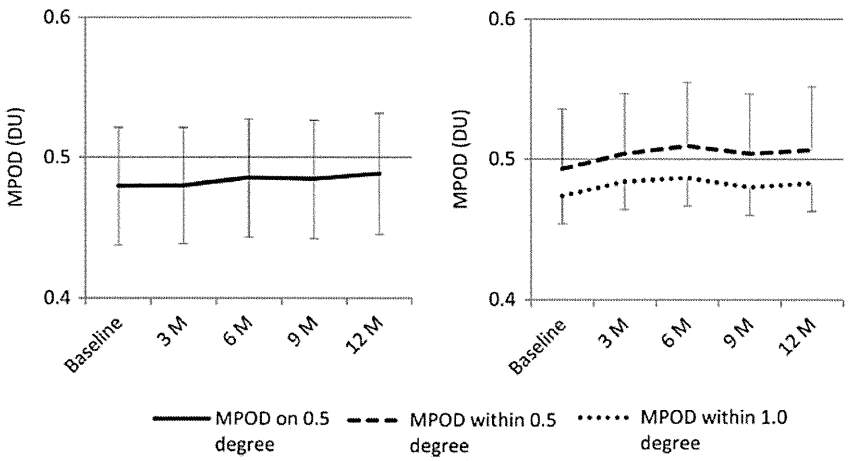

Fig. 1 Time-dependent changes in the mean MPOD levels on 0.5 degree (**a**) and within 0.5 and 1.0 degree (**b**). There were no significant differences between baseline and 3, 6, 9, and 12 months in those areas. *MPOD* macular pigment optical density; *DU* density unit; *M* months

The low-contrast VA and the AULCSF were measured every 3 months (Fig. 2). There were no significant differences between baseline and 3, 6, 9, and 12 months in the low-contrast VA (p=0.9051, p=0.6446, p=0.5598, and p=0.5556, respectively). Although there were also no significant differences between baseline and 3, 6, and 9 months in the AULCSF (p=0.3060, p=0.8850, and p=0.7151, respectively), the AULCSF was significantly (p=0.0124) higher at 12 months than at baseline (1.36, 95% CI, 1.32, 1.40 versus 1.41, 95% CI, 1.37, 1.45).

We also analyzed the differences between baseline and 6 and 12 months in the mean retinal sensitivity (Fig. 3). The retinal sensitivities within 0.5 degree were 16.2 decibels (dB) (95% CI, 15.3, 17.2) at 6 months and 17.0 dB at 12 months (95% CI, 16.0, 18.0), which were significantly higher than at baseline, 14.8 dB (95% CI, 13.9, 15.8) (p<0.0001, p<0.0001, respectively). Similarly, the retinal sensitivity within 6.0 degrees was 16.5 dB (95% CI, 15.8, 17.2) at 6 months and 17.2 dB (95% CI, 16.4, 18.0) at 12 months, and they were significantly higher than at baseline, 15.3 dB (95% CI, 14.6, 16.0) (p<0.0001, p<0.0001, respectively).

We performed stepwise regression analyses to determine the covariates that correlated with the 1-year changes in factors representative of visual function (Table 2). As a result, the increase in the low-contrast VA correlated with subjects with no cardiovascular diseases (T=2.14, p=0.0386) and the lower baseline low-contrast VA (T=−3.18, p=0.0029). The increase in the AULCSF correlated with the nonsmoking (T=2.49, p=0.0172), the phakic eye (T=2.16, p=0.0372) and the lower baseline AULCSF (T=−3.97, p=0.0003). The increase in the retinal sensitivity within 0.5 degree correlated with the phakic eye (T=2.42, p=0.0202) and the lower baseline retinal sensitivity within 0.5 degree (T=−2.92, p=0.0058), and the lower increase of the retinal sensitivity within 6.0 degrees correlated with the fellow eye of the eye with CSC (T=−2.03, p=0.0491)

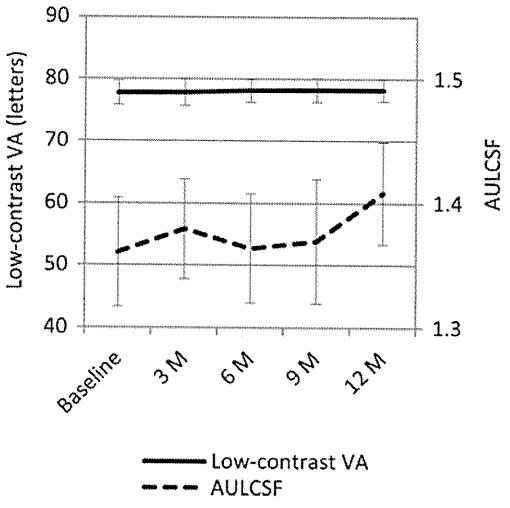

Fig. 2 Time-dependent changes in the low-contrast VA and the AULCSF. There were no significant differences between baseline and 3, 6, 9, and 12 months in the low-contrast VA. Although there were also no significant differences between baseline and 3, 6, and 9 months in the AULCSF, the AULCSF was significantly higher at 12 months than at baseline (p=0.0124). *VA* visual acuity; *AULCSF* area under the log contrast sensitivity function; *M* months

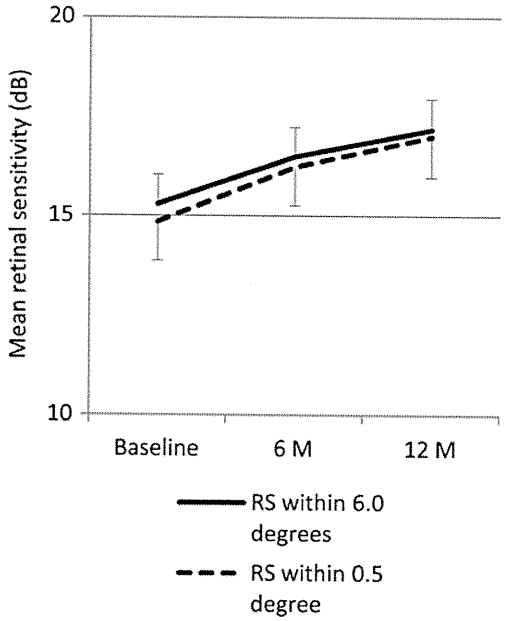

Fig. 3 Time-dependent changes in the retinal sensitivity. The retinal sensitivities within 0.5 degree at 6 months and 12 months were significantly higher than at baseline (p<0.0001, p<0.0001, respectively). Similarly, the retinal sensitivity within 6.0 degrees at 6 months and 12 months were significantly higher than at baseline (p<0.0001, p<0.0001, respectively). *RS* retinal sensitivity; *dB* decibels; *CI* confidence interval

Discussion

Supplementation with lutein may prevent development and/or progression of AMD because carotenoids help prevent antioxidative stress [3–9]. Whether lutein supplementation increases the MPOD level is now intensely discussed. Most studies have shown that lutein positively affects the MPOD level, but usually the increase in the MPOD level is not high [20–25]. However, a greater increase in the MPOD is likely to be correlated with higher doses of lutein [27, 28]. Previous studies in which a lutein dose under 10 mg was used reported no significant increase in the MPOD level, although the serum level of lutein increased [26].

In the current study, we evaluated the effect of supplementation with 6 mg of lutein, vitamins, and minerals on the MPOD level and the relationship with visual function in Japanese subjects. The 43 subjects who

Table 2 Selected covariates in the increased visual function

Visual function	Selected variable	T value	*p* value
Low-contrast VA	No cardiovascular disease	2.14	0.0386
	CSC in the fellow eye	−1.78	0.0822
	Baseline low-contrast VA	−3.18	0.0029
AULCSF	Non smoking	2.49	0.0172
	Phakic eye	2.16	0.0372
	Baseline AULCSF	−3.97	0.0003
Retinal sensitivity within 0.5 degrees	Phakic eye	2.42	0.0202
	Baseline retinal sensitivity within 0.5 degrees	−2.92	0.0058
Retinal sensitivity within 6.0 degrees	CSC in the fellow eye	−2.03	0.0491

VA visual acuity; *AULCSF* area under the log contrast sensitivity function; *CSC* central serous chorioretinopathy

took supplementation containing 6 mg of lutein were followed for 1 year and the MPOD level and visual function were measured every 3 or 6 months. We followed all subjects for 1 year, which satisfied the condition of the number needed for the primary outcome measure. As affected eyes with CSC and AMD had some foveal abnormalities when observed by fundus autofluorescence spectrometry and the measured MPOD levels were unstable, we did not include any affected eyes in the current study.

At baseline, we found that the eyes with clear IOL had higher MPOD levels. This agrees with our previous report that showed the MPOD level measured by autofluorescence spectrometry with two-wavelength autofluorescence method become higher after cataract surgery [45].

After 1 year of follow-up, the MPOD levels did not increase significantly, but it was stable throughout the study period and no covariates were significant for the changes in the MPOD levels. The LUtein Nutrition effects measured by Autofluorescence (LUNA) study in Germany, in which subjects with normal eyes or AMD took a supplement containing 12 mg of lutein, 1 mg of zeaxanthin, 120 mg of vitamin C, 17.6 mg of vitamin E, 10 mg of zinc, and 40 μg of selenium for 6 months, reported a significant increase in the MPOD level in the intervention group [24, 25]. Because the LUNA study used the same autofluorescence methods to measure the MPOD as we used in the current study, the different responses in MPOD seemed to be due to the different amounts of supplemental lutein. Although this difference may also be influenced by the racial difference, number of tested subjects and the amount of other antioxidants, 6 mg of lutein supplementation may be too low to increase the MPOD level. On the other hand, it is possible that the development of cataract might affect the measurement of MPOD since the decrease of the MPOD level within 1.0 degree correlated with the phakic eye. This theory is also supported by our previous report that showed a higher nuclear color grading score was correlated with a lower MPOD level measured by autofluorescence spectrometry with two-wavelength autofluorescence method [45]. Although we did not observe the apparent decrease of visual acuity during follow-up period, cataract might develop and mask the increase of MPOD induced by lutein supplementation. In addition, the 1-year increase in the MPOD level was correlated with subjects with no cardiovascular diseases. As previous reports showed that the cardiovascular risk factors are associated with AMD, subjects with cardiovascular diseases may have less response to lutein supplementation [46, 47].

The contribution of macular pigments to visual performance has been reported. Macular pigments can improve visual performance including contrast sensitivity by reducing chromatic aberration, blue haze, and the intensity of the rod signal, for example [28–36]. To identify the minute changes in visual contrast, we measured the low-contrast VA and contrast sensitivity [40]. The low-contrast VA was correlated with aging at baseline and did not change during the follow-up period; therefore, antioxidants may not affect the low-contrast VA. However, the AULCSF, which is a representative index for evaluating the contrast sensitivity, significantly increased at 12 months (p=0.0124). Supplementation containing lutein may have maintained the MPOD level and improved the contrast sensitivity. Interestingly, the increase in AULCSF was correlated with nonsmoking status, although the AULCSF was not correlated with smoking at baseline. Uz et al. reported that smoking reduced contrast sensitivity possibly because of the decreased serum level of the trace elements manganese and zinc [35]. Because the supplement we used in the current study contained manganese and zinc, they may have caused the contrast sensitivity to improve especially in nonsmokers. Contrary to our results, Bartlett and Eperjesi reported no correlation between contrast sensitivity and supplementation with 6 mg of lutein, vitamins, and minerals [29]. The relationship between antioxidant supplement and contrast sensitivity should be investigated further.

Retinal sensitivity was measured by microperimetry within 0.5 and 6.0 degrees. The baseline retinal sensitivity within 0.5 and 6.0 degrees was correlated with aging as reported previously [48, 49]. The retinal sensitivity significantly increased during the follow-up period. To the best of our knowledge, this is the first report to show a correlation between supplementation with antioxidants containing lutein and the retinal sensitivity. Because retinal sensitivity is measured by a white target on a white background by changing the light intensity of the target, there may be some mechanisms other than macular pigment playing roles in contrast sensitivity; one hypothesis is that antioxidants may improve the light threshold of the photoreceptors. Stepwise regression analysis showed that the fellow eyes with CSC had a lower increase of retinal sensitivity within 6.0 degrees. These results might be associated with lower MPOD in the fellow eyes of those with CSC, which have a high risk of developing CSC [15]. On the other hand, stepwise regression analysis revealed that the subjects with cardiovascular diseases showed lower increase in the low-contrast VA, and the subjects with clear IOL showed lower change of AULCSF and retinal sensitivity within 0.5 degree. The reasons for these results are hard to explain at the moment, possibly because the number of the subgroup is too small. Accordingly, further studies are needed on those points.

In conclusion, although there is a fear that the autofluorescence spectrometry with two-wavelength method may not be appropriate to detect MPOD augmentation in elderly eyes, daily supplementation with 6 mg of lutein, vitamins, and minerals did not affect the MPOD level measured by this method for 1 year. Supplementation of antioxidants containing 6 mg of lutein may be insufficient to increase the MPOD level, however, it may improve visual function such as contrast sensitivity and retinal sensitivity. So far, we cannot deny the possibility that the improved visual function was due to a learning effect, because our study had no placebo control group. Further studies may provide deeper insights into the role of antioxidant supplementation in maintaining the function of macula.

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Intravitreal bevacizumab for exudative branching vascular networks in polypoidal choroidal vasculopathy

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ABSTRACT

Aims To assess the long-term efficacy of intravitreal bevacizumab for recurrent leakage owing to the residual branching vascular networks in polypoidal choroidal vasculopathy after photodynamic therapy.

Methods Forty-five eyes with exudative branching vascular networks were treated with intravitreal bevacizumab and followed for at least 24 months. Original polypoidal lesions had been treated successfully with previous photodynamic therapy in all eyes. The best-corrected visual acuity and retinal morphological changes were assessed retrospectively.

Results Exudative branching vascular networks were characterised as occult choroidal neovascularisation (38 eyes) or classic choroidal neovascularisation (7 eyes) on fluorescein angiography. Intravitreal bevacizumab maintained or improved vision in 38 eyes (84%) over 12 months and in 36 eyes (80%) over 24 months, although the mean visual acuity at 12 and 24 months did not differ significantly compared with baseline. Complete resolution of macular fluid was achieved continuously in 26 eyes (58%) during 24 months. Sixteen eyes (36%) responded once to treatment but became unresponsive to additional injections for recurrent exudation. Three eyes (7%) were refractory to treatment throughout follow-up. Cystoid macular oedema eventually developed in 10 eyes and was a poor prognostic sign for visual outcome.

Conclusion Intravitreal bevacizumab improved the retinal morphology and maintained vision over 1 year in most eyes with recurrent fluid owing to persistent abnormal vascular networks in polypoidal choroidal vasculopathy. The therapeutic response, however, may decrease during the second year.

Polypoidal choroidal vasculopathy (PCV), characterised by a complex of branching vascular networks terminating in polypoidal lesions,[1–5] accounts for 23–54% of neovascular age-related macular degeneration (AMD) in Asian populations.[6 7] Photodynamic therapy (PDT) maintains or improves vision by resolving the polypoidal lesions and accompanying fluid beneath the neurosensory retina.[8–10] However, the branching vascular networks usually remain even after PDT,[11–13] and may enlarge further over time, resulting in persistent exudation appearing as choroidal neovascularisation (CNV) secondary to AMD in some cases.[14] Therefore, stabilisation of the branching vascular networks may be crucial for long-term management of PCV.

Recent studies have reported the efficacy and safety of bevacizumab (Avastin, Genentech, South San Francisco, California) for stabilising neovascular activity and maintaining vision in patients with neovascular AMD.[15] Those results promoted us to consider the drug as the treatment of choice for CNV-like branching vascular networks of PCV after PDT.

The purpose of this retrospective study is to assess the long-term efficacy of intravitreal bevacizumab in the management of exudative branching vascular networks in eyes with PCV.

METHODS

This study was a retrospective, consecutive, interventional case series conducted at Osaka University Hospital. Patients who received intravitreal bevacizumab for the treatment of recurrent exudation associated with branching vascular networks between December 2006 and April 2008 and followed for at least 24 months were initially enrolled. All eyes had a previous history of successful PDT for PCV with resolution of polypoidal lesions. Recurrent exudation was determined by the presence of subretinal fluid (SRF) or macular oedema on optical coherence tomography (OCT) with CNV-like fluorescein leakage caused by branching vascular networks. Patients were excluded if they had SRF or macular oedema caused by recurrent polypoidal lesions, follow-up <24 months after intravitreal bevacizumab or clinically relevant media opacity.

All patients underwent a comprehensive ocular examination, including measurement of the best-corrected visual acuity (BCVA), intraocular pressure, binocular indirect ophthalmoscopy and contact lens slit-lamp biomicroscopy, colour fundus photography, OCT, fluorescein angiography (FA) and indocyanine green angiography (ICGA). The patients were examined after a detailed explanation of the study was provided, and they provided informed consent. This study was approved by the institutional review board committee of Osaka University Hospital.

ICGA analysis

ICGA was performed at baseline before intravitreal bevacizumab and 3–6 months and 12–24 months after intravitreal bevacizumab. A confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2 (HRA2), Heidelberg Engineering GmbH, Dossenheim, Germany) was used as previously described.[14] The planimetric size of the branching vascular networks was measured from the early-phase ICGA at baseline to the final visits using software included in the HRA2.[14] The individual readings of two investigators were averaged.

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A change in lesion size was recorded when the lesion increased or decreased more than 50% in the corresponding area.

OCT analysis

OCT images were obtained by Stratus OCT (Carl Zeiss Meditec, Dublin, California) or the Cirrus HD-OCT (Carl Zeiss Meditec). The central retinal thickness (CRT), defined as the distance between the internal limiting membrane and the inner surface of the retinal pigment epithelium (RPE), was measured manually at the fovea.[16] The SRF and intraretinal fluid were included in the CRT measurements, whereas sub-RPE fluid was not included. The fluid in the macula was identified as intraretinal fluid (macular oedema) and SRF, and a fluid-free macula was defined by the absence of macular oedema and SRF as determined by OCT.

Follow-up and reinjection protocols

Intravitreal bevacizumab (1.25 mg) was injected during an outpatient procedure under strict aseptic conditions. All patients were followed monthly for more than 24 months. The VA and OCT were examined at every visit. Re-treatment with intravitreal bevacizumab was considered if there was OCT evidence of macular fluid with at least one-line loss of VA, new macular haemorrhage or newly developed fibrinous changes. A treatment response on OCT was defined as complete if there was no macular fluid, partial if there was no macular fluid initially but the lesions become refractory to treatment after recurrence of exudation, or no response if there was not an absence of macular fluid. When recurrence was suspected, FA and ICGA were performed at the discretion of the physician.

Data collection and statistical analysis

The main outcome measures were the changes in exudative fluid and the BCVA during 24 months after the initial injection. The changes in the CRT and the size of the branching vascular networks before and after injection were also evaluated. Statistical analyses were performed with SAS software, version 9.1 (SAS Institute). P-values <0.05 were considered significant.

RESULTS

Forty-five eyes of 45 patients met the criteria for data analysis. The patient demographics are shown in table 1. Original polypoidal lesions had resolved in all eyes on ICGA with complete absence of fluid after previous PDT, but the branching vascular network remained in all eyes. The development of subsequent exudative changes associated with residual branching vascular networks without recurrent polypoidal lesions was seen 9.3±8.4 months (range 1–36) after the previous PDT (figure 1).

Table 1 Patient baseline characteristics

No eyes	45
No patients	45
Age (years) (mean±SD; range)	70.4±6.9 (53–83)
Gender (no/%)	
Men	35 (78)
Women	10 (22)
Eye (no/%)	
Right	20 (44)
Left	25 (56)
Interval between intravitreal bevacizumab and previous photodynamic therapy (mean±SD; range)	9.3±8.4 (1–36)
Optical coherence tomography characteristics	
Subretinal fluid (no/%)	43 (96)
Pigment epithelial detachment (no/%)	26 (58)
Macular oedema (no/%)	10 (22)
Leakage pattern on fluorescein angiography	
Classic choroidal neovascularisation	7 (16)
Occult choroidal neovascularisation	38 (84)
Baseline best-corrected visual acuity	
Landolt C acuity chart (mean; range)	0.35 (0.09–1.2)
Logarithm of the minimum angle of resolution (mean±SD)	0.45±0.30

Angiographic and OCT characteristics of exudative branching vascular networks

FA showed leakage mimicking occult CNV in 38 eyes (84%) (figure 1C,G) and classic CNV in seven eyes (16%) (figure 1K). Of the 38 eyes with occult CNV-like lesions, ICGA showed thin branching choroidal vessels that depicted relatively well-delineated plaque in the late phase. Of the seven eyes with classic CNV-like lesions, subretinal fibrinous exudation was seen in all eyes. The exudation associated with the branching vascular networks was characterised by OCT as SRF in 43 eyes (96%) and macular oedema in 10 eyes (22%). Apparent PED was seen in 24 eyes (53%), and the limited RPE elevation was detected in 14 eyes (31%).

Visual acuity, ICG angiography and OCT outcomes

The mean number of injections in the 45 eyes during the first 12 months was 2.9±1.8 (range 1–8) and 1.9±1.8 (range 0–6) during the second year.

The mean baseline BCVA was 0.45±0.30 (table 1). The mean BCVA values were 0.43±0.33, 0.43±0.30, 0.48±0.32 and 0.51±0.38 at 3, 6, 12 and 24 months, respectively ($p=0.118$, $p=0.428$, $p=0.523$ and $p=0.206$, respectively) (figure 2A). The BCVA at the 12-month follow-up visit improved by three or more lines in four eyes (9%), was unchanged within three lines in 34 eyes (76%) and worsened in seven eyes (16%). At 24 months, BCVA improved by three or more lines in six eyes (13%), was unchanged in 30 eyes (67%) and worsened in nine eyes (20%).

ICGA images showed persistent branching vascular networks in all 45 eyes during follow-up. The size of the branching vascular network increased in 20 eyes (44%), remained unchanged in 21 eyes (47%) and decreased in four eyes (9%) at the final ICGA examination compared with baseline. In six eyes, polypoidal lesions reappeared at the site connected to the branching vascular networks. The period between the initial intravitreal bevacizumab and detection of newly developed polypoidal lesions was 3 months in one eye, 6 months in one eye and 24 months in four eyes.

The mean baseline CRT measured on OCT was 222±71 μm (range 87–434). The CRT decreased an average of 31.0 μm from baseline by 3 months ($p<0.001$) and an average of 40.0 μm by 6 months ($p<0.001$) (figure 2B). Those initial decreases lessened over time after initial intravitreal bevacizumab, with an average reduction of 25.8 μm by 12 months ($p=0.050$) and 1.8 μm by 24 months ($p=0.296$). There tended to be a correlation between the CRT at 24 months and the BCVA at 24 months ($r=0.292$, $p=0.051$).

A fluid-free macula was achieved during the first 12 months in 42 eyes (93%). The mean number of injections required to achieve a fluid-free macula was 1.9±1.6 (range 1–9). Subsequently, 36 eyes developed recurrent macular fluid and received additional injections. Overall, 26 eyes (58%) were regarded on OCT as complete responders because intravitreal bevacizumab was effective throughout the 24 months of follow-up in completely resolving the exudation. One initial injection was effective to keep the macula dry for 24 months in two eyes.

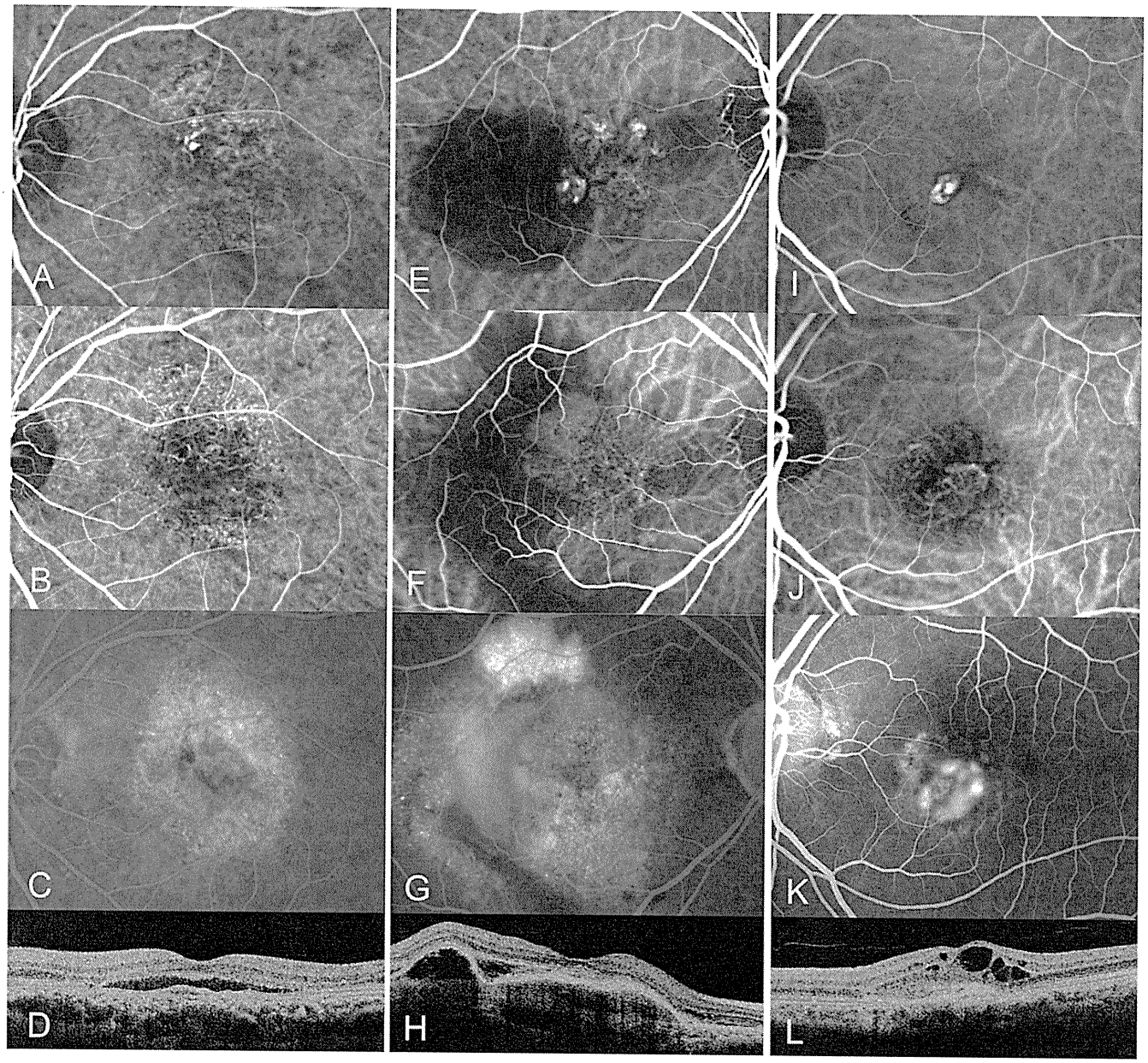

Figure 1 Exudation associated with branching vascular networks in eyes with polypoidal choroidal vasculopathy. The exudation patterns are characterised as occult choroidal neovascularisation (CNV) without apparent pigment epithelial detachment (PED) (A—D), occult CNV with fibrovascular PED (E—H) or classic CNV (I—L). (A) Indocyanine green angiography (ICGA) before previous photodynamic therapy (PDT) showing branching vascular networks terminating in small polypoidal lesions in a 64-year-old man. The polypoidal lesions resolved completely after PDT. However, the branching vascular networks remained (B) and subsequently developed exudative changes resembling occult CNV on fluorescein angiography (FA) 6 months after the previous PDT (C). (D) Optical coherence tomography (OCT) showing subretinal fluid (SRF) with limited retinal pigment epithelium elevation. (E) ICGA before previous PDT showing branching vascular networks terminating in polypoidal lesions with a serous PED in a 77-year-old man. The polypoidal lesions have resolved completely after PDT. However, the branching vascular networks have enlarged (F) and subsequently developed exudative changes resembling occult CNV with a fibrovascular PED on FA 3 months after previous PDT (G). (H) OCT showing a large PED with SRF. (I) ICGA before previous PDT showing small branching vascular networks terminating in polypoidal lesions in a 57-year-old woman. The polypoidal lesions have resolved completely after PDT. Without recurrence of the polypoidal lesion, the branching vascular networks show exudative changes at the macula (J) resembling classic CNV on FA 6 months after previous PDT (K). (L) The OCT shows fibrin accumulation and macular oedema.

Sixteen eyes (36%) were considered to be partial responders. Those eyes once had no macular fluid after injection during the first 12 months; however, they became unresponsive to treatment despite repeated injections for recurrent exudation (figure 3). Three eyes (7%) were considered non-responders because the macular fluid persisted during 24 months despite repeated injections. Eight of 16 eyes with partial responses and two of three eyes with no response eventually developed cystoid macular oedema (CMO), and six of these lost three lines or more at 24 months compared with baseline.

The differences between complete responders and partial or non-responders with respect to BCVA and OCT findings are shown in table 2. Although the baseline BCVA, the leakage pattern on FA, CRT and the lesion size did not differ significantly

Br J Ophthalmol 2012;**96**:394—399. doi:10.1136/bjo.2011.204123

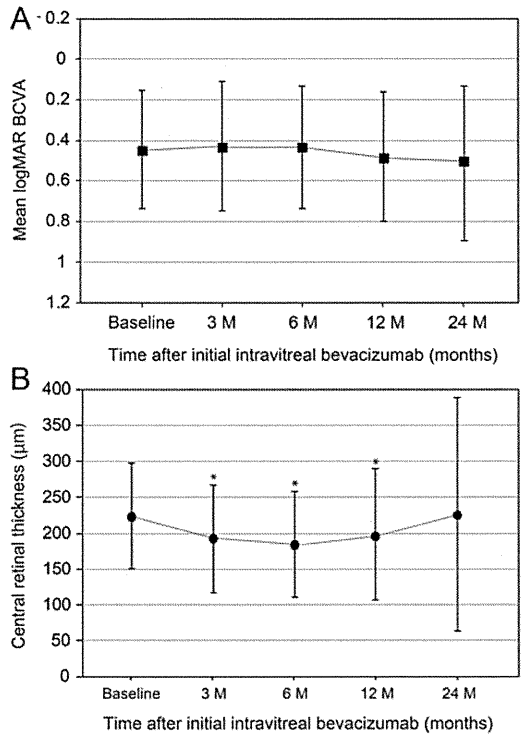

Figure 2 (A) Mean change in the best-corrected visual acuity (BCVA) (mean±SD) after intravitreal bevacizumab for exudative branching vascular networks through 24 months. The visual acuity is expressed as the logarithm of the minimum angle of resolution (logMAR). There are no statistical differences between the baseline and post-treatment BCVA values at any time point. (B) Mean change in the central retinal thickness (CRT) (mean±SD) after intravitreal bevacizumab for exudative branching vascular networks through 24 months. The mean CRT has decreased significantly at 3, 6 and 12 months after initial intravitreal bevacizumab (*p<0.05 compared with baseline). However, there are no statistical differences in CRT between baseline and 24 months. M, months.

between the groups, the mean BCVA in eyes with a complete response was significantly better than in eyes with a partial or no response at 24 months (p=0.041). However, there was no significant difference in the BCVA between eyes with a complete response and those with partial responses or no response at 24 months when the 10 eyes with CMO were excluded from the analysis (0.41±0.34 vs 0.34±0.30, p=0.675). The CRT also differed significantly between the groups at 24 months (p=0.001). The branching vascular networks were significantly larger in eyes with a partial response or no response compared with those who were complete responders at 24 months (p=0.049).

No adverse systemic and local complications related to intravitreal bevacizumab were observed during the study period. One eye developed an RPE tear.

DISCUSSION

In the current study, we focused on identifying the characteristics of the exudative features associated with branching vascular networks and assessed the potential efficacy of bevacizumab to treat those lesions. We found two angiographic patterns of exudative branching vascular networks at an average of 9.3±8.4 months (range 1–20) after previous PDT, that is, occult CNV-like lesions with or without fibrovascular PED (84%) and classic CNV-like leaky vascular lesions (16%).

In eyes with occult CNV-like leakage, the complex of abnormal vessels was clearly seen on early-phase ICGA, with hyperfluorescent plaques on late-stage ICGA. On OCT, the lesion was also identifiable as elevated RPE, indicating invasion of the networks beneath the RPE. Thus, PCV in this stage was clinically and angiographically indistinguishable from type 1 neovascularisation. Imamura and associates also indicated that it would be difficult to differenciate between PCV and CNV unless typical polypoidal lesion exists in the network vessels.[17] Serous and haemorrhagic PEDs associated with PCV before PDT seemed to be potentially predictive of the development and progression of occult CNV-like exudative vessels, because in those eyes, the rate of PED at the time of PDT was 63% and was higher than that reported previously in PCV (19–44%).[7 8 18] In eyes with classic CNV-like lesions, OCT showed subretinal fibrin formation, indicating extensive exudation. Both the occult and classic CNV-like lesions in our study did not seem to have the same characteristics as the original branching vascular networks of PCV, which had previously been considered as long-term stable abnormal choroidal vessels.[18 19] We speculate that pre-existing branching vascular networks acquired hyperpermeable properties mimicking CNV or the CNV newly emerged from residual branching vascular networks over time after PDT.

Intravitreal bevacizumab for exudative branching vascular networks maintained or improved vision in 38 eyes (84%) over 12 months, although the mean VA at 12 months did not differ significantly compared with baseline. The significant decrease in retinal thickness was achieved during the first year due to complete resolution of macular fluid in 42 (93%) eyes, indicating the potent effect of bevacizumab on antipermeability in most eyes over 12 months.

Improved or stabilised VA was continuously achieved in 36 eyes (80%) over 24 months. According to a previous study of patients with PCV treated only by PDT for 3 years, VA deteriorated three lines or more in 37%, owing to the enlargement of abnormal networks with neovascular changes and recurrent polyps.[20] In contrast to these reports, the current results using bevacizumab are encouraging because VA deterioration was seen in 20%, despite a mean follow-up period of 33 months after initial PDT.

Complete resolution of macular fluid was maintained throughout the study in 58% of eyes (complete responders). However, 36% of eyes that responded to treatment during the first 12 months showed less treatment effect, despite repeated injections for recurrent exudation (partial responders). Three eyes were considered non-responders on OCT, and the macular fluid increased further. As a result, the decrease in the mean retinal thickness during the first year lessened during the second year and became non-significant (p=0.296) at 24 months compared with baseline.

The loss of the therapeutic response to bevacizumab during 2 years may be explained by a potential change in the sensitivity to bevacizumab. In the current study, the vascular networks persisted on ICGA in all eyes after intravitreal bevacizumab and even enlarged in 20 eyes (44%) despite repeated injections. Continuous growth of the CNV despite repeated anti-vascular endothelial growth factor therapy also had been reported in eyes with AMD.[21] The persistence and expansion of the networks may lead to more mature and less vascular-endothelial-growth-factor-dependent vessels with increased treatment resistance. In addition, a tachyphylactic response, that is, a progressive decrease in the bioefficacy of bevacizumab, after repeated injections has been reported in AMD.[22] Those responses also

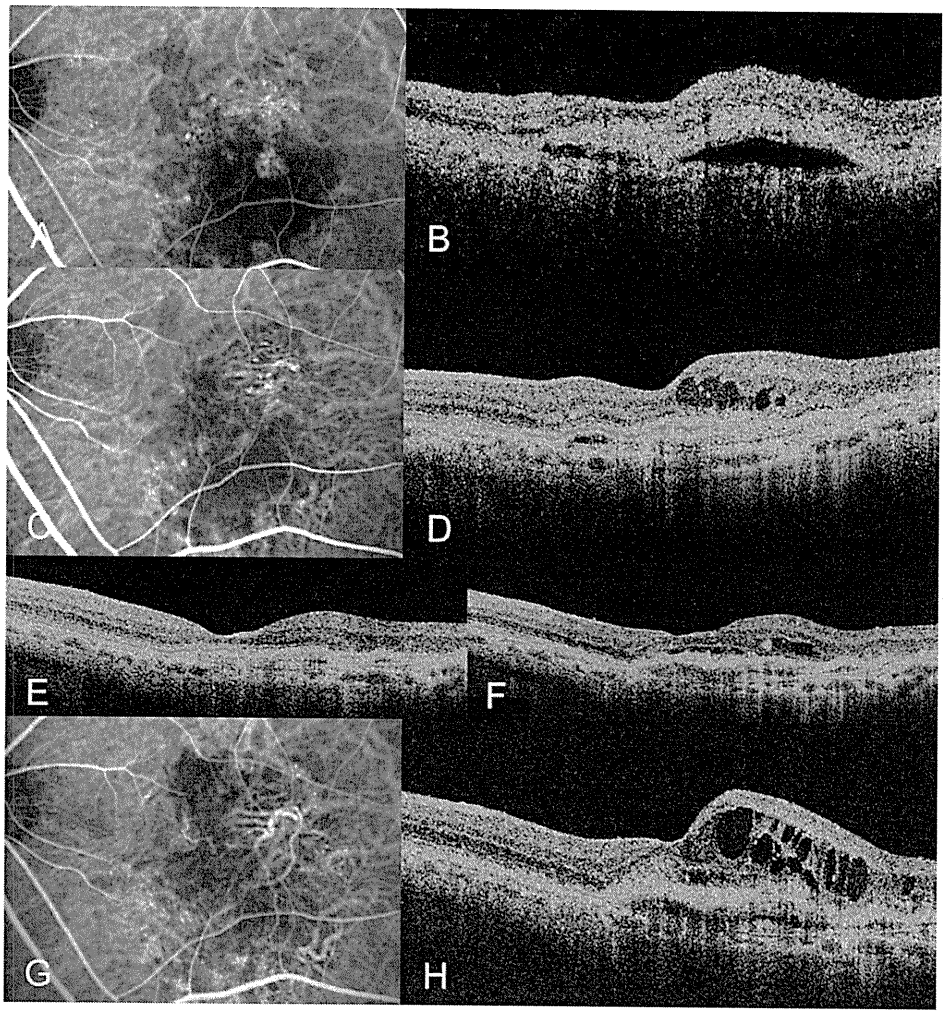

Figure 3 Indocyanine green angiography (ICGA) and optical coherence tomography (OCT) images before and after intravitreal bevacizumab in an 83-year-old man. (A) ICGA showing branching vascular networks terminating in polypoidal lesions with a serous pigment epithelial detachment (PED) before previous photodynamic therapy (PDT). (B) OCT image confirming the PED. The polypoidal lesions have resolved completely after PDT. However, the branching vascular networks remain with subsequently developing exudation 6 months after previous PDT (C). (D) OCT showing slight subretinal fluid (SRF), macular oedema and a fibrovascular PED. The visual acuity (VA) is 0.2. Intravitreal bevacizumab was administered. After intravitreal bevacizumab, the SRF and macular oedema have resolved on the OCT image (E). The VA is 0.3. However, OCT shows recurrent macular oedema 3 months after the first injection (F). The VA is 0.2. Additional intravitreal bevacizumab was administered, and the SRF and macular oedema resolved again. The macular oedema recurred once again and was successfully treated with repeated injections during the first year. Fifteen months after the initial injection, the branching vascular networks have increased in size (G). Despite repeated intravitreal bevacizumab for recurrent exudation, the SRF and macular oedema that had once responded to treatment during the first year persistently remained during the second year with deterioration of VA to 0.1, indicating loss of the treatment efficacy (H).

Table 2 Differences between complete responders and partial or non-responders in best-corrected visual acuity and optical coherence tomography findings

	Complete responders (n=26)	Partial or non-responders (n=19)	p Value
Age (years), mean±SD (range)	70.5±6.5 (57–83)	70.4±7.6 (53–83)	0.985
Patterns of leakage on fluorescein angiography at baseline			
Classic choroidal neovascularisation	5	1	
Occult choroidal neovascularisation	21	18	0.222
Baseline optical coherence tomography findings			
Pigment epithelial detachment (no/%)	15 (58)	11 (58)	0.770
Subretinal fluid (no/%)	25 (96)	18 (95)	1.000
Macular oedema (no/%)	5 (19)	5 (26)	0.720
Best-corrected visual acuity, logarithm of the minimum angle of resolution±SD			
Baseline	0.43±0.29	0.48±0.32	0.583
12 months	0.40±0.31	0.58±0.32	0.058
24 months	0.41±0.34	0.66±0.40	0.041
Logarithm of the minimum angle of resolution change between baseline and 24 months	0.02±0.37	−0.18±0.27	0.047
Central retinal thickness (μm), mean±SD			
Baseline	219±60 (87–337)	226±85 (128–434)	0.738
12 months	172±56 (80–347)	229±115 (128–664)	0.017
24 months	159±54 (76–288)	313±216 (113–834)	0.001
Change between baseline and 24 months	−61±81 (−215–118)	87±216 (−246–678)	0.003
Branching vascular network size (mm^2)			
Baseline	4.59±2.72 (1.18–9.98)	5.52±4.23 (0.88–16.86)	0.705
24 months	6.30±3.53 (1.14–15.90)	11.39±10.01 (2.85–45.23)	0.049

Br J Ophthalmol 2012;**96**:394–399. doi:10.1136/bjo.2011.204123

may be responsible for the loss of treatment efficacy in the current study eyes.

Fortunately, half of the eyes that were partial and non-responders maintained the VA through 24 months despite persistent macular fluid. However, 10 eyes eventually developed significant CMO with poor visual outcomes. The CMO has been reported to be associated with all forms of neovascular AMD, including classic CNV, occult CNV, PED and disciform scars,[23 24] or with PCV.[20] We speculated that the persistent exudative vascular networks beneath the RPE may cause RPE decompensation, resulting in severe damage to the neurosensory retina, as seen in progressed AMD.

The limitations of the current study were its retrospective nature and the absence of a control group. Because the individual responses vary among the patients, we did not re-treat patients at fixed intervals but did so with the criteria based on their responses under monthly monitoring. Some patients with a lower morphological response despite consecutive injections were not always re-treated unless VA declined, as reported in the treatment of AMD.[25] To confirm whether the current strategy is optimal for long-term follow-up, a prospective, randomised, comparative study should be considered comparing different injection strategies for exudative branching vascular networks in PCV.

In summary, the intravitreal bevacizumab to treat exudative branching vascular networks in patients with PCV improves the retinal morphology and maintains vision over 1 year. However, the network vessels persist, and the therapeutic response may be lost during the second year. The visual prognosis is poor in eyes with CMO. Further studies may elucidate the appropriate use of intravitreal bevacizumab or other treatment modalities for better management of branching vascular networks in PCV.

Competing interests None.

Patient consent Obtained.

Ethics approval Ethics approval was provided by the institutional review board committee of Osaka University Hospital.

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Intravitreal bevacizumab for exudative branching vascular networks in polypoidal choroidal vasculopathy

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Long-term efficacy and safety of ranibizumab administered *pro re nata* in Japanese patients with neovascular age-related macular degeneration in the EXTEND-I study

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ABSTRACT.

Purpose: **To evaluate the long-term efficacy and safety of ranibizumab administered *pro re nata* (PRN) in Japanese patients with choroidal neovascularization secondary to age-related macular degeneration during the extension phase of the EXTEND-I study.**

Methods: **EXTEND-I, an open-label, multicenter, Phase I/II study comprised: a single-injection (Group A); a multiple-injection (Groups A and B; the latter consisted of patients who did not participate in the single-injection phase); and an extension phase. In the extension phase, a PRN regimen of ranibizumab (0.3 or 0.5 mg) guided by monthly best-corrected visual acuity (BCVA) score and other ophthalmic examinations was employed. The efficacy variables included the mean BCVA change from Month 12 to the last visit in Group B. Safety was assessed in all patients.**

Results: **In the extension phase, efficacy was assessed only in Group B patients. The number of ranibizumab injections per year in the 0.3 and 0.5 mg Group B patients was 4.19 and 4.27, respectively. The mean BCVA change (SD) from Month 12 to the last visit was −3.6 (14.82) letters for 0.3 mg (n = 28) and −2.2 (7.92) letters for 0.5 mg groups (n = 33) in Group B. Conjunctival haemorrhage and nasopharyngitis were the most commonly reported adverse events. Of the 13 serious adverse events reported, cerebral infarction (two incidences) was suspected to be study-drug related.**

Conclusions: ***Pro re nata*** **regimen of ranibizumab guided by monthly BCVA and other ophthalmic examinations appears effective in sustaining the BCVA gained with 12 monthly injections while reducing the number of injections during the extension phase. Ranibizumab was well tolerated during the extension phase.**

Key words: age-related macular degeneration – best-corrected visual acuity score – efficacy – individualized flexible interval regimen – Japanese patients – PRN – ranibizumab – safety – subfoveal choroidal neovascularization

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Introduction

Age-related macular degeneration (AMD) is a leading cause of vision loss in the elderly population. Of the two types of AMD, the wet form caused by choroidal neovascularization (CNV) is mainly responsible for AMD-related vision loss (Bressler 2004). According to the Hisayama study (prospective cohort study in Japan), the prevalence of neovascular AMD in residents aged 50 years or older was 0.67% in 1998, which was lower than that observed in the Caucasians (Oshima et al. 2001). However, another recent study (The Funagata study) in Japanese residents aged 35 years or older suggested that the prevalence of neovascular AMD in Japanese men was similar to that seen in the Caucasian men (Kawasaki et al. 2008).

Current evidence points to the role of vascular endothelial growth factor (VEGF) in CNV proliferation, and hence agents that block its activity are considered as a suitable therapeutic intervention in the management of this form of AMD (Ferrara et al. 2006; Waisbourd et al. 2007). Ranibizumab (Lucentis®; Novartis Pharma AG, Basel, Switzerland and Genentech Inc, South San Francisco, CA, USA)

is a humanized monoclonal antibody fragment that inhibits active forms of VEGF-A, the main factor responsible for CNV proliferation and vascular permeability (Ferrara et al. 2006, 2007). Benefits of ranibizumab treatment in improving best-corrected visual acuity (BCVA) have been shown in the Caucasian population (Brown et al. 2006, 2009; Rosenfeld et al. 2006; Mitchell et al. 2010). Ranibizumab is currently approved in the United States, European Union, Japan and several other countries. EXTEND-I was the first study in Japanese patients that showed the safety and efficacy of monthly ranibizumab treatment (12-month results) during multiple-injection phase in terms of BCVA gain, reduction in total area of leakage from CNV plus retinal pigment epithelium staining and foveal retinal thickness, which were consistent with the pivotal studies performed in the Caucasian population (Tano & Ohji 2010). After the patients had completed the 12-month multiple-injection phase, all patients who provided written consent and were eligible based on the inclusion and exclusion criteria of the extension phase had the opportunity to continue to receive the 'individualized flexible interval regimen' [namely, *pro re nata* (PRN), as needed] until the approval of ranibizumab in Japan. This also provided a means to assess its long-term safety and efficacy. The PRN regimen was expected to maintain the improved visual acuity (VA) with less frequent injections in the extension phase. Current treatment guidelines in Europe recommend three initial monthly dosing followed by a maintenance phase, wherein the ranibizumab administration is decided based on monthly BCVA observation (Holz et al. 2010; Mitchell et al. 2010). This recommendation is based mainly on the results of the ranibizumab pivotal randomized phase III studies, namely MARINA (Rosenfeld et al. 2006) and ANCHOR (Brown et al. 2006) with monthly ranibizumab treatment. In these studies, the improvement of the BCVA score had stabilized (almost reached a plateau) by Month 3, and further increase in BCVA was minimal during the subsequent monthly treatments. On the other hand, in another pivotal randomized Phase IIIb study, PIER, quarterly treatment regimen could not maintain the improvement in BCVA score that was obtained by the three initial monthly injections (Regillo et al. 2008). However, there were also patients who maintained their gain in BCVA score during the quarterly regimen.

The extension phase of this study was initiated, therefore, to investigate whether ranibizumab administered PRN based on monthly BCVA scores and other ophthalmic examinations at two consecutive visits could maintain the improvement in BCVA scores. The reduction in dosing frequency was expected to reduce the risk of adverse events (AEs) associated with the intravitreal injection procedure in the elderly population as well as to address the difficulties in treating AMD through monthly injection of ranibizumab in a clinical setting.

Based on the 6-month interim results of the extension phase with PRN regimen as well as the 6- and 12-month interim analyses of monthly multiple-injection phase of this study, and the results of pivotal studies in the Caucasian population, ranibizumab was approved in Japan in January 2009. This paper presents the final data on long-term efficacy (in terms of BCVA) and safety of ranibizumab with PRN regimen from whole period of the extension phase of EXTEND-I.

Methodology

Study design

EXTEND-I was an open-label, multicentre, Phase I/II study comprising three phases: a single-injection phase, a multiple-injection phase and an extension phase (Fig. 1). The single-injection phase (Group A) was designed to sequentially evaluate the safety of intravitreal injections of 0.3 and 0.5 mg ranibizumab (six patients treated with each dose). The patients who successfully completed the single-dose phase (i.e., did not experience a Grade-3 targeted AE) could enter a multiple-injection phase wherein they received the same dose for an additional 11 months. The 12-month multiple-injection phase (Groups A and B; the latter consisted of patients who did not partic-

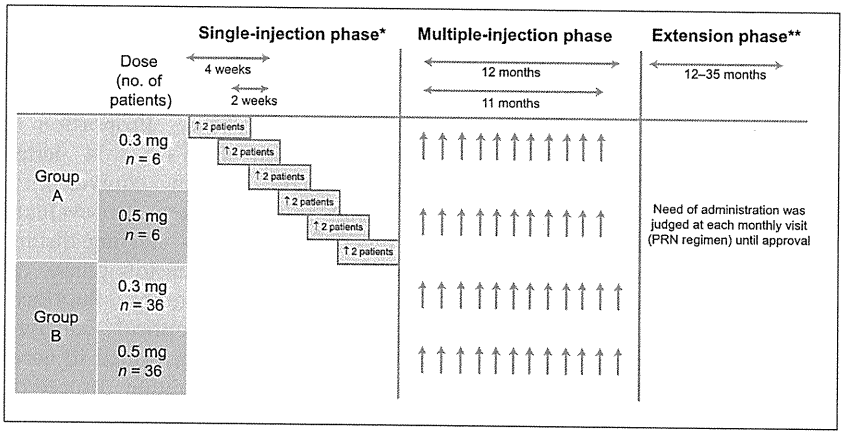

Fig. 1. EXTEND-I treatment schedule. *Upon completion of the single-dose phase, patients in Group A were eligible to enter the multiple-injection phase, which began ≥4 weeks after the final visit of the single-injection phase. Multiple injections did not begin until both doses were shown to be well tolerated in all cohorts. **Upon completion of the multiple dose phase, based on prespecified inclusion/exclusion criteria, patients could enter the extension phase. For the extension phase, treatment as per *pro re nata* regimen; dose same as core phase; retreatment at the monthly visit if loss of >5 letters in best-corrected visual acuity (BCVA) on two consecutive visits (except unscheduled visits), considering other ophthalmic examinations, such as slit-lamp examination, ophthalmoscopy, fundus photography, fluorescein angiography and optical coherence tomography, the investigator decided whether ranibizumab treatment would be performed. Similarly, if the BCVA score decreased on two consecutive visits (except unscheduled visits) by ≤5 letters using ETDRS-like visual acuity chart, a decision was taken whether treatment could be withheld. In any case, other ophthalmic examinations were taken into consideration. For the extension phase, the number of patients for Group A was 3 in the 0.3 mg group and 6 in the 0.5 mg group; the number of patients for Group B was 28 in the 0.3 mg group and 33 in the 0.5 mg group.

ipate in the single-injection phase) evaluated the safety and efficacy of both doses administered as monthly intravitreal injections in two parallel groups of 0.3 mg dose and 0.5 mg dose (Tano & Ohji 2010). The multiple-injection phase was followed by an extension phase in which the ranibizumab (0.3 or 0.5 mg) administration was on a PRN basis, but assessments were carried out on a monthly basis. If the BCVA score decreased at two consecutive visits (except unscheduled visits) by >5 letters, considering other ophthalmic examinations, such as slit-lamp examination and ophthalmoscopy for safety, fundus photography, fluorescein angiography and optical coherence tomography for efficacy, the investigator decided whether ranibizumab treatment would be administered although no specific retreatment criteria were provided for fundus photography, fluorescein angiography and optical coherence tomography and were at the discretion of the investigators. Similarly, if the BCVA score decreased at two consecutive visits (except unscheduled visits) by ≤5 letters, in conjunction with other ophthalmic examinations, a decision was taken whether the treatment could be withheld.

This study was conducted in accordance with the Declaration of Helsinki, International Conference on Harmonization Good Clinical Practice (GCP) guidelines and Japanese GCP. The study was approved by Institutional Review Boards at each study centre. All patients provided written informed consent before participating in the study and the extension. The trial is registered with clinicaltrials.gov (NCT00275821).

Inclusion and exclusion criteria

All patients with subfoveal CNV secondary to AMD who completed the multiple-injection phase in either of the ranibizumab groups (Groups A or B), provided written consent and met all of the inclusion criteria set at the beginning of the study (Tano & Ohji 2010) were eligible to enrol in the extension phase. Patients were allowed to participate in the extension phase regardless of the time elapsed between the exit visit of the multiple-injection phase and the participation in the extension phase.

Patients were excluded from the extension phase if they had received any anti-angiogenic drugs (bevacizumab, pegaptanib, ranibizumab, anecortave acetate, corticosteroids or protein kinase C inhibitors) or participated in any other clinical study of an investigational drug during the period from the exit visit of the multiple-injection phase to participation in the extension phase. However, as the extension phase was not started on the day of the exit visit from the multiple-injection phase, photodynamic therapy with verteporfin was allowed for the study eye during the transition period.

Efficacy assessments

The efficacy variables of the extension phase included mean change from Month 12 in BCVA score of the study eye using ETRDS chart (at a starting distance of 2 m) at the last visit of the extension phase for Group B patients only. Group A patients were not included as they were not assessed for efficacy, but only for safety throughout the study. The other efficacy variables included the proportion of patients at the last visit with a BCVA score loss <15 letters, and ≥30 letters, or a BCVA score gain of ≥15 letters in the study eye. Proportion of patients with BCVA <34 letters, approximate Snellen equivalent of 20/200 or worse, were also evaluated (ETDRS charts at a starting distance of 2 m). In the extension phase, colour fundus photography, fluorescein angiography and optical coherence tomography were performed in accordance with the routine procedures specified at each study site.

Safety assessments

All safety evaluations were based on the enrolled population (Groups A and B) of the extension phase. Safety assessments consisted of recording the frequency of the treatment collecting all AEs, serious adverse events (SAEs), with their severity, and relationship to study drug. It also included monitoring of haematology, serum chemistry, urinalysis and regular assessments of vital signs. Grade 3 targeted AEs (Tano & Ohji 2010), intraocular inflammation, myocardial infarction and stroke and AEs potentially related to systemic VEGF inhibition were analysed separately. Serum samples for the evaluation of immunoreactivity to ranibizumab (antiranibizumab antibodies) were obtained from patients prior to study administration at Month 23 and the last visit for Group A patients, and Month 24 and the last visit for Group B patients. At the last visit as well as at early termination, the assessments were performed if at least 6 months had passed since the previous measurement, on or after Month 11 for Group A patients and Month 12 for Group B patients. The last measurement in the multiple-injection phase of the study was performed at Month 11 for Group A and Month 12 for Group B.

Statistical analysis

The patient population included all enrolled patients in the extension phase. This population was used for all analyses in Groups A and B. All efficacy data presented were for observed cases without the last observation carried forward method.

Descriptive statistics of the number of injections, duration of exposure and reason of injection were presented for the enrolled population. The duration of treatment varied for each patient in the extension phase. To reduce a possible bias because of the patients who discontinued early without injection, the number of injections per year was calculated as 365.25 × sum of total number of injections in the group/duration of the PRN regimen for the respective group. The number of injections per year was calculated for the respective group and not per patient. Duration of the PRN regimen was the date of the last potential treatment visit minus the date of Month 11 visit (the last treatment visit of multiple-injection phase) plus 1.

All efficacy analyses were based on the study eye. Descriptive statistics (mean, median, standard deviation, standard error, minimum and maximum) of the change from baseline (the single-injection phase of Group A and the multiple-injection phase of Group B), Month 11 and Month 12 in Group B were performed by treat-

ment and visit. The 95% confidence intervals based on *t*-distributions and p-values based on paired *t*-tests were determined for the change from baseline. Exact 95% confidence intervals were calculated for the proportion of patients with the specified response rates.

Results

Patients

Overall, 70 patients at 11 sites participated in the extension phase from 20 March 2007 to 20 January 2009: 9 in Group A (3 and 6 in the 0.3 and 0.5 mg dose groups, respectively) and 61 in Group B (28 and 33 in the 0.3 and 0.5 mg dose groups, respectively) as shown in Table 1. In Group A, a total of seven patients were not discontinued in the extension phase. Two patients in the 0.3 mg dose group withdrew from the study, as their condition did not further require the study drug. In Group B, 22 patients in the 0.3 mg dose group and 21 patients in the 0.5 mg dose groups were not discontinued in the extension phase. Six patients in the 0.3 mg dose group and 12 patients in the 0.5 mg dose group withdrew from the extension study. The maximum number of patients discontinued as they did not require the study drug because of improvement in VA (n = 9, two in the 0.3 mg dose group and seven in the 0.5 mg dose group); other reasons being AEs (n = 4, two in each dose group), withdrawal of consent (n = 4, one in the 0.3 mg dose group and three in the 0.5 mg dose group) and protocol violation (n = 1, one in the 0.3 mg dose group). None of the AEs leading to study discontinuation was thought to be related to the study drug.

The mean duration of treatment (standard deviation, SD) during the extension phase was 1.70 (0.35) years in the 0.3 mg group and 1.93 (0.09) years in the 0.5 mg dose group in Group A (Table 1). In Group B patients, the mean duration of treatment was 1.45 (0.33) years and 1.36 (0.39) years in the 0.3 and 0.5 mg dose groups, respectively.

The baseline demographic and ocular characteristics of enrolled patients at the start of the extension phase are given in Table 2. The mean (SD) BCVA score of the study eye at the start of the extension phase was 59.1 (11.69) letters and 59.8 (15.07) letters in the 0.3 and 0.5 mg dose groups of Group B, respectively. Overall, approximate Snellen equivalent VA of almost all patients was better than 20/200 except for two patients in the 0.5 mg dose group.

Of the 61 patients in Group B, approximately 90% (25/28 and 27/33 in the 0.3 mg and the 0.5 mg dose groups, respectively, Table 3) completed Month 24 from the baseline of the multiple-injection phase of the study, i.e., these patients received treatment of ranibizumab with PRN for 12 months in the extension phase. The duration of treatment of each patient in the extension phase varied with respect to the study entry and the longest was 35 months from baseline for the 0.3 mg dose group (n = 1). For the 0.5 mg dose group, the longest was 34 months (n = 1), as shown in Fig. 2.

Table 1. Patient disposition in the extension phase.

Disposition/patients studied	Group A Ranibizumab 0.3 mg	Group A Ranibizumab 0.5 mg	Group B Ranibizumab 0.3 mg	Group B Ranibizumab 0.5 mg
Patients (n %)				
Enrolled	3 (100.0)	6 (100.0)	28 (100.0)	33 (100.0)
Not discontinued	1 (33.3)	6 (100.0)	22 (78.6)	21 (63.6)
Discontinued	2 (66.7)	0 (0.0)	6 (21.4)	12 (36.4)
Main cause of discontinuation				
Adverse event (s)	0 (0.0)	0 (0.0)	2 (7.1)	2 (6.1)
Patient's condition does not requires study drug	2 (66.7)	0 (0.0)	2 (7.1)	7 (21.2)
Protocol violation	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Patient withdrew consent	0 (0.0)	0 (0.0)	1 (3.6)	3 (9.1)
Mean duration, years, of the extension phase (SD)	1.70 (0.35)	1.93 (0.09)	1.45 (0.33)	1.36 (0.39)

Table 2. Baseline demographics of enrolled patients and ocular characteristics (study eye) at the start of the extension phase.

Characteristic	Category/statistic	Group A Ranibizumab 0.3 mg N = 3	Group A Ranibizumab 0.5 mg N = 6	Group B Ranibizumab 0.3 mg N = 28	Group B Ranibizumab 0.5 mg N = 33
Gender – n (%)	Male	3 (100.0)	5 (83.3)	19 (67.9)	28 (84.8)
	Female	0 (0.0)	1 (16.7)	9 (32.1)	5 (15.2)
Age, years	Mean (SD)	68.0 (10.15)	72.0 (4.82)	69.8 (8.72)	70.2 (7.83)
Race (%)	Asian	3 (100.0)	6 (100.0)	28 (100.0)	33 (100.0)
Best-corrected visual acuity score	Mean (SD)	72.0 (4.58)	58.5 (15.66)	59.1 (11.69)	59.8 (15.07)
	Range	68–77	42–77	39–80	36–85
Approximate Snellen equivalent n (%)	Median	40.0	70.0	71.5	63.0
	20/200 or worse	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.1)
	Better than 20/200 but worse than 20/40	1 (33.3)	3 (50.0)	20 (71.4)	20 (60.6)
	20/40 or better	2 (66.7)	3 (50.0)	8 (28.6)	11 (33.3)
Intraocular pressure (mmHg)	Mean (SD)	13.3 (1.53)	14.2 (3.06)	13.5 (2.92)	13.7 (3.09)
	Range	12–15	9–18	8–20	9–23

Data of ocular characteristics are based on Month 11 visit in Group A and Month 12 visit in Group B.
N = number of enrolled patients, n = number of patients.

Table 3. Summary of patient exposure to ranibizumab for 12 months (from Month 12 to Month 24) in the extension phase (Group B, enrolled patients).

Cumulative number of injections	Ranibizumab 0.3 mg (N = 28)	Ranibizumab 0.5 mg (N = 33)
Month 24		
n	25	27
Mean (SD)	4.1 (4.12)	3.9 (4.63)
Range	0–13	0–13
0	7	9
1–2	3	8
3–6	9	2
7–9	2	3
10–12	3	4
13	1	1
Number of injections per Year	4.19	4.27

The number of injections per year is calculated as: 365.25 × total number of injections/duration of the *pro re nata* (PRN) regimen.
Number of injections per year is calculated for total group, not per patient.
Duration of the PRN regimen: date of last potential treatment visit – date of Month 11 visit + 1.
N = number of enrolled patients, n = number of patients.

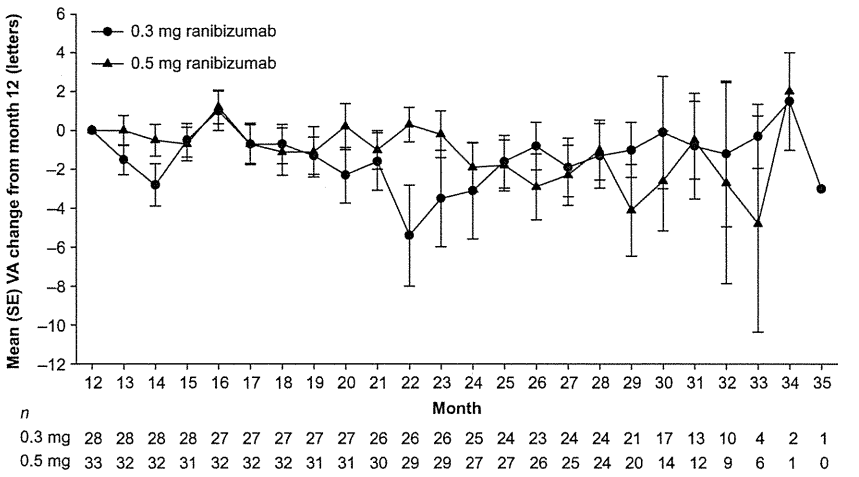

Fig. 2. Mean change from Month 12 (the start of Extension phase) in best-corrected visual acuity score (±SE) of study eye by visit during extension phase (Group B patients).

The exposure to ranibizumab in the extension phase of Group B is shown in Table 3. At Month 24, the patients had been treated with the PRN regimen for 12 months in the extension phase, and hence the maximum achievable number of injections by this visit was 13. The injection frequency of ranibizumab for individual patient varied from 0 to 13 times for this 12 months in the extension phase. The estimated number of injections per year in the extension phase was 4.19 and 4.27 in the 0.3 and 0.5 mg dose groups in Group B, respectively.

Efficacy

The mean change (SD) from Month 12 in BCVA score of the study eye to the last visit in the extension phase was −3.6 (14.82) letters in the 0.3 mg group and −2.2 (7.92) letters in the 0.5 mg group of Group B using the PRN regimen (Table 4). Furthermore, the mean change (SD) from baseline in BCVA score of the study eye to the last visit in the extension phase was 7.5 (19.12) letters in the 0.3 mg group and 7.7 (13.02) letters in the 0.5 mg group (p = 0.0475 for the 0.3 mg dose group and p = 0.0019 for the 0.5 mg dose group) (Table 4). Overall, the improvement in BCVA score at Month 12 by monthly ranibizumab injection was sustained throughout the extension phase with the PRN regimen (Fig. 2).

Table 5 shows the proportion of patients with respect to VA outcome at the last visit in the extension phase. The proportion of patients who lost <15 letters from baseline in BCVA in the study eye was 85.7% (24/28) and 97.0% (32/33) in the 0.3 and 0.5 mg dose groups, respectively. Nine patients each in the 0.3 mg (32.1%) and 0.5 mg (27.3%) dose groups gained ≥15 letters from the baseline. One patient each in the 0.3 mg (3.6%) and 0.5 mg (3.0%) dose groups lost ≥30 letters from the baseline. The proportion of patients with approximate Snellen equivalent of 20/200 or worse was 14.3% (4/28) and 6.1% (2/33) in the 0.3 and 0.5 mg groups, respectively.

The mean time to first retreatment in the extension phase since Month 11 of the multiple-injection phase (when the last monthly injection was done) in Group B was 218.5 days (range: 29–512 days) for the 0.3 mg group and 255.6 days (range: 29–571 days) for the 0.5 mg group.

Safety

In Group A patient population (n = 9), two of three (66.7%) patients in the 0.3 mg dose group and three of six (50.0%) patients in the 0.5 mg dose group experienced at least one ocular AE in the study eye during the extension phase. In Group B, 20 of 28 (71.4%) patients in the 0.3 mg dose group and 18 of 33 (54.5%) patients in the 0.5 mg dose group experienced at least one ocular AE in the study eye during the extension phase. The most common ocular AE in the study eye in Group B was conjunctival haemorrhage. Other frequent ocular AEs included retinal haemorrhage, retinal detachment and increased intraocular pressure (Table 6). Two patients in the 0.3 mg dose group of Group B experienced Grade 3 targeted AEs (intraocular inflammation, reduced VA, increased intraocular pressure, vitreous haemorrhage, retinal tear or detachment, and retinal haemorrhage). One patient experienced retinal detachment, retinal haemorrhage and vitreous haemorrhage in the study eye, and the other patient experienced retinal haemorrhage in the fellow eye.

One patient in the 0.3 mg dose group of Group B experienced iritis in the study eye among the ocular AEs defined under the group of intraocular inflammation (iritis, iridocyclitis, vitritis, uveitis, hypopyon and anterior chamber inflammation). Two kinds of ocular AEs in six patients of Group B were suspected to be study-drug related: increased intraocular pressure (two patients in the 0.3 mg dose group and three patients in the 0.5 mg dose group) and retinal haemorrhage (one patient in the 0.5 mg dose group).