Choroidal thickness after intravitreal ranibizumab injections for choroidal neovascularization

Abdallah A Ellabban
Akitaka Tsujikawa
Ken Ogino
Sotaro Ooto
Kenji Yamashiro
Akio Oishi
Nagahisa Yoshimura

Department of Ophthalmology and Visual Sciences, Kyoto University Graduate School of Medicine, Kyoto, Japan

Purpose: To study changes in choroidal thickness with ranibizumab treatment for choroidal neovascularization (CNV).

Design: Prospective case series.

Methods: This prospective study consisted of 60 CNV-affected eyes of 60 patients treated with intravitreal injections of ranibizumab using an on-demand protocol after an initial loading phase. The eyes studied included 20 with age-related macular degeneration (AMD), 20 with polypoidal choroidal vasculopathy (PCV), and 20 with myopic CNV. In the eyes with AMD and PCV, choroidal thickness at the fovea was measured with optical coherence tomography using enhanced depth imaging. In eyes with myopic CNV, the choroidal thickness was measured using standard optical coherence tomography without the enhanced depth imaging technique.

Results: With ranibizumab treatment, central retinal thickness decreased significantly (*P*, 0.001) and visual acuity improved significantly (*P*, 0.001). However, central choroidal thickness (167.2 ± 108.3 µm) showed no significant change at 1 month after the loading phase (165.2 ± 107.8 µm, *P* = 0.120) or at final examination (164.8 ± 107.7 µm, *P* = 0.115). At baseline, central retinal thickness in eyes with AMD was significantly greater than those with PCV (*P* = 0.005) or high myopia (*P* = 0.029). However, central choroidal thickness in eyes with myopic CNV was significantly thinner than in eyes with AMD (*P* < 0.001) or PCV (*P* < 0.001). In each type of disease, there was no significant change in central choroidal thickness with ranibizumab treatment.

Conclusion: The effect of ranibizumab on the choroidal thickness is minimal, if any.

Keywords: choroidal thickness, ranibizumab, optical coherence tomography

Introduction

Ranibizumab, a Fab fragment of a recombinant, humanized, monoclonal antibody, blocks all isoforms of vascular endothelial growth factor (VEGF)-A. The inhibition of VEGF-A reduces the leakage from choroidal neovascularization (CNV) and further growth of CNV. The current standard treatment for exudative age-related macular degeneration (AMD) is intravitreal injections of ranibizumab. Previous large-scale, prospective, randomized studies of eyes with AMD showed dramatic effects of ranibizumab on CNV and subsequent improvement of visual acuity. However, most patients with AMD require frequent injections of ranibizumab to maintain the initial visual improvement during the loading phase. Mendrinos et al recently reported that the retinal arteriolar diameter was reduced by 18.5% at 30 days and by 19.1% at 12 months after the initiation of ranibizumab treatments for exudative AMD. Because VEGF has various physiological effects on eyes, including cell proliferation, neuroprotective...
effects, and maintenance of the choriocapillaris, and increases in ocular blood flow, long-term inhibition of VEGF may adversely affect the eye.

The choroid is a multifunctional structure with one of the highest blood flow rates in the body. It primarily works to support the function and nutrition of the outer retina, including photoreceptors and retinal pigment epithelium. Since Spaide et al introduced enhanced depth-imaging optical coherence tomography (EDI-OCT), which is based on spectral-domain OCT technology, many investigators have reported on the role of the choroid in the pathogenesis of various ocular diseases, including AMD, polypoidal choroidal vasculopathy (PCV), central serous chorioretinopathy, and myopic CNV. It has recently been suggested that choroidal thinning may be involved in the development of CNV associated with AMD and high myopia. VEGF works to induce vessel dilation and to increase ocular blood flow through a mechanism involving increased nitric oxide production. It is then possible that long-term inhibition of VEGF by ranibizumab might cause the constriction of the choroidal vessels, leading to further thinning of the choroid. Thus far, however, only limited information is available on the effects of ranibizumab on the choroid. In this prospective study, we performed longitudinal measurements of choroidal thickness before and after ranibizumab treatment for CNV associated with AMD, PCV, and high myopia to evaluate the possible adverse effects of ranibizumab on the choroid.

**Patients and methods**

This prospective study consisted of 60 eyes of 60 patients with CNV treated with intravitreal injections of ranibizumab (Lucentis; Novartis, Bülach, Switzerland). The choroidal thickness of the eyes was examined using OCT at Kyoto University Hospital between May 2010 and August 2011. The underlying pathology of the 60 eyes included 20 consecutive eyes with AMD, 20 with PCV, and 20 with myopic CNV. The inclusion criteria for the study groups were (1) symptomatic neovascular lesions beneath the center of the fovea, (2) the presence of active exudative features involving the macula, (3) treatment by intravitreal injections of ranibizumab after the initial diagnosis, and (4) OCT examination before and after treatments. Exclusion criteria were (1) the obscuration of retinal or choroidal images by media opacity or thick subfoveal hemorrhage, (2) a history of intraocular inflammation, (3) the presence or history of other macular abnormalities (eg, idiopathic CNV, retinal angiomatous proliferation, angiod streaks, other secondary CNV, or central serous chorioretinopathy), (4) a history of previous treatment for CNV, and (5) eyes with a history of pars plana vitrectomy or other intraocular surgeries, except history of cataract surgery. Subjects with systemic diseases or conditions that could affect retinal or choroidal thickness were also excluded, such as those with Vogt-Koyanagi-Harada disease, malignant hypertension, or pregnant women.

At the initial visit, each patient underwent a comprehensive ophthalmologic examination, including measurement of best-corrected visual acuity with a Landolt chart; determination of intraocular pressure; indirect opthalmoscopy; slit lamp biomicroscopy with a contact lens; OCT examination (Spectralis HRA+OCT; Heidelberg Engineering, Heidelberg, Germany); and fluorescein and indocyanine green angiography (HRA-2; Heidelberg Engineering). The diagnostic criteria for PCV were based on indocyanine green angiography, which showed a branching vascular network that terminates in polypoidal swelling. The diagnosis of myopic CNV was based on a minimum spherical equivalent refractive error of more than −6.0 diopters or an axial length of more than 26.5 mm, characteristic retinal signs of high myopia, and evidence of CNV based on the presence of leakage on fluorescein angiography and/or intraretinal or subretinal fluid on OCT.

At the loading phase, all eyes with PCV and AMD received three successive intravitreal injections of ranibizumab at monthly intervals (at baseline and at 1 and 2 months); eyes with myopic CNV were treated by a single injection of ranibizumab. After the loading phase, each patient was scheduled for an examination each month, at which time they underwent a comprehensive ophthalmologic examination. Each eye was re-treated by an intravitreal injection of ranibizumab as an on-demand protocol, depending on the presence of exudative signs, such as intraretinal edema or subretinal fluid on OCT and/or leakage on fluorescein angiography during follow-up. Injections of ranibizumab were performed under sterile conditions, and prophylactic topical antibiotics were applied for 1 week after the injection.

In eyes with AMD and PCV, the choroidal thickness was measured using the EDI technique, which was performed by placing the OCT instrument close enough to the eye to obtain an inverted image. All images were obtained using an eye-tracking system, and 100 scans were averaged automatically to improve the signal-to-noise ratio. The central choroidal thickness, defined as the vertical distance between the outer surface of the retinal pigment epithelium and the hyperreflective line of the choriocapillaris interface, was measured from the horizontal line scan under the center of the fovea.
myopic CNV, the choroidal thickness was measured with standard OCT without the EDI technique, as identification of the choriocapillaris and choroidal thickness were performed using SPSS version 16.0 software (SPSS Inc, Chicago, IL). All values are presented as mean ± standard deviation. For statistical analysis, best-corrected visual acuity as measured with a Landolt chart was converted to a logarithm of the minimal angle of resolution. Initial measurement values between the three types of diseases were compared using one-way analysis of variance with Bonferroni corrections. Measurements before and after the treatments were analyzed by repeated measures analysis of variance, and multiple comparisons were done using the Bonferroni post-hoc method. A P value of <0.05 was considered statistically significant.

Table 1 Characteristics of cohort eyes

<table>
<thead>
<tr>
<th>Total</th>
<th>Age-related macular degeneration</th>
<th>Polypoidal choroidal vasculopathy</th>
<th>Myopic choroidal neovascularization</th>
<th>P value&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of eyes</td>
<td>60</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>71.1 ± 9.6</td>
<td>76.2 ± 6.2</td>
<td>71.7 ± 9.2</td>
<td>65.6 ± 10.3</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>36/24</td>
<td>15/5</td>
<td>16/4</td>
<td>5/15</td>
</tr>
<tr>
<td>Refractive error (diopters)</td>
<td>−2.74 ± 5.97</td>
<td>1.54 ± 1.75</td>
<td>−0.56 ± 1.28</td>
<td>−10.96 ± 3.32</td>
</tr>
<tr>
<td>(range)</td>
<td>(−21.0–3.0)</td>
<td>(−1.75–3.0)</td>
<td>(−2.75–2.5)</td>
<td>(−21.0–7.0)</td>
</tr>
<tr>
<td>Number of injections</td>
<td>3.2 ± 1.4</td>
<td>3.5 ± 0.9</td>
<td>3.9 ± 1.4</td>
<td>2.2 ± 1.3</td>
</tr>
<tr>
<td>(range)</td>
<td>(1–7)</td>
<td>(3–6)</td>
<td>(3–7)</td>
<td>(1–5)</td>
</tr>
<tr>
<td>Follow-up (months)</td>
<td>8.9 ± 3.9</td>
<td>8.4 ± 3.7</td>
<td>8.4 ± 3.3</td>
<td>9.8 ± 4.6</td>
</tr>
<tr>
<td>Visual acuity (logMAR)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45 ± 0.37</td>
<td>0.61 ± 0.41</td>
<td>0.24 ± 0.23</td>
<td>0.49 ± 0.37</td>
</tr>
<tr>
<td>Central retinal thickness (μm)</td>
<td>436.0 ± 198.5</td>
<td>551.0 ± 232.5</td>
<td>361.2 ± 161.4</td>
<td>395.9 ± 143.9</td>
</tr>
<tr>
<td>Central choroidal thickness (μm)</td>
<td>167.2 ± 108.3</td>
<td>217.4 ± 98.6</td>
<td>230.8 ± 68.8</td>
<td>52.6 ± 38.2</td>
</tr>
</tbody>
</table>

Notes: *LogMAR, logarithm of the minimum angle of resolution; <sup>c</sup>values were compared using one-way analysis of variance with Bonferroni corrections.
Discussion

An increasing number of investigators have studied the choroid in association with the pathophysiology of various chorioretinal diseases.17–24,29,34,35 With the use of EDI-OCT, Chung et al23 and Koizumi et al24 separately reported foveal choroidal thinning in eyes with exudative AMD. In addition, with the use of 3-dimensional OCT at 1060 nm, Wood et al25 reported choroidal thinning of the macular area in eyes with early AMD. The thinning of the macular choroid may be partially involved in the development of CNV associated with AMD. In eyes with high myopia, previous reports have shown that the choroid is extremely thin;29,30 the central choroidal thickness in our patients with high myopia was 53.6 ± 38.1 μm, which was much thinner than in patients with AMD and PCV. In addition, Ikuno et al31 reported that eyes with myopic CNV have a thinner choroid than their contralateral eye without CNV; the authors speculated that a thinner choroid is a risk factor for CNV in eyes with high myopia.23,24,31

Treatment with ranibizumab is expected to induce long-term inhibition of all isoforms of VEGF-A in the eye.1 It may well be that physicians are concerned about the adverse effect of ranibizumab on the choroid, especially when they treat eyes with AMD or high myopia. In the current study, choroidal thickness (167.2 ± 108.3 μm) showed no significant change after the loading phase (165.2 ± 107.8 μm) or at final examination (164.8 ± 107.7 μm). In a PubMed search, we could find no reference to changes in choroidal thickness after anti-VEGF treatment. Based on a retrospective analysis of 47 eyes with exudative AMD treated with ranibizumab, Sadda et al37 have recently noted that choroidal thickness appears to decrease slightly over time in eyes with exudative AMD, but that ranibizumab does not appear to accelerate this decline. Previously, Margolis and Spaide15 reported that subfoveal choroidal thickness decreased by 1.56 μm per year in normal subjects. Our patients showed a mean decrease of 2.4 μm (1.4%) in central choroidal thickness during a mean follow-up time of 8.9 months. This decrease in choroidal thickness may not be so different from the change seen in normal subjects.

More recently, Koizumi et al38 reported that in eyes with exudative AMD treated with ranibizumab, mean subfoveal choroidal thickness decreased from 228 μm at baseline to 213 μm at 3 months (P = 0.001), 215 μm at 6 months (P = 0.009), and 213 μm at 12 months (P = 0.015). Although the authors found a statistically significant decrease in the foveal choroidal thickness, the mean reduction was only 15 μm. Recently, Ikuno et al39 reported that the intervisit intraclass correlation coefficient for foveal choroidal thickness was 0.893 (95% CI, 0.864–0.916). However, because measurement of choroidal thickness has high variance due to focal irregularities or indistinctness of the chorioscleral border,23,24,40,41 it might be difficult to detect minimal changes in thickness practically, even with the EDI-OCT technique. Based on the current findings and other recent reports, the effect of ranibizumab on choroidal thickness is estimated to be small, if any.

In contrast to AMD23,24 and high myopia,29,30 previous reports showed increased choroidal thickness in PCV.23,24,26

### Table 2 Changes in visual acuity, retinal thickness, and choroidal thickness during ranibizumab treatment

<table>
<thead>
<tr>
<th></th>
<th>Before treatment</th>
<th>After the loading phase&lt;sup&gt;c&lt;/sup&gt;</th>
<th>At final examination&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Visual acuity (logMAR)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.45 ± 0.37</td>
<td>0.33 ± 0.38 (0.005)</td>
<td>0.28 ± 0.34 (&lt;0.001)</td>
</tr>
<tr>
<td>Age-related macular degeneration</td>
<td>0.61 ± 0.41</td>
<td>0.58 ± 0.43 (0.663)</td>
<td>0.45 ± 0.33 (0.030)</td>
</tr>
<tr>
<td>Polypoidal choroidal vasculopathy</td>
<td>0.24 ± 0.23</td>
<td>0.19 ± 0.29 (0.885)</td>
<td>0.12 ± 0.25 (0.046)</td>
</tr>
<tr>
<td>Myopic choroidal neovascularization</td>
<td>0.49 ± 0.37</td>
<td>0.23 ± 0.28 (&lt;0.001)</td>
<td>0.24 ± 0.31 (&lt;0.001)</td>
</tr>
<tr>
<td><strong>Central retinal thickness (μm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>436.0 ± 198.5</td>
<td>262.9 ± 113.8 (&lt;0.001)</td>
<td>256.7 ± 106.0 (&lt;0.001)</td>
</tr>
<tr>
<td>Age-related macular degeneration</td>
<td>551.0 ± 232.5</td>
<td>298.4 ± 156.6 (&lt;0.001)</td>
<td>293.2 ± 147.8 (&lt;0.001)</td>
</tr>
<tr>
<td>Polypoidal choroidal vasculopathy</td>
<td>361.2 ± 161.4</td>
<td>244.7 ± 92.2 (0.008)</td>
<td>236.2 ± 76.1 (0.004)</td>
</tr>
<tr>
<td>Myopic choroidal neovascularization</td>
<td>395.9 ± 143.9</td>
<td>245.7 ± 72.1 (&lt;0.001)</td>
<td>240.8 ± 71.7 (&lt;0.001)</td>
</tr>
<tr>
<td><strong>Central choroidal thickness (μm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>167.2 ± 108.3</td>
<td>165.2 ± 107.8 (0.120)</td>
<td>164.8 ± 107.7 (0.115)</td>
</tr>
<tr>
<td>Age-related macular degeneration</td>
<td>217.4 ± 98.6</td>
<td>214.2 ± 99.2 (0.305)</td>
<td>215.1 ± 98.7 (0.586)</td>
</tr>
<tr>
<td>Polypoidal choroidal vasculopathy</td>
<td>230.8 ± 68.8</td>
<td>228.7 ± 68.0 (0.876)</td>
<td>227.4 ± 67.9 (0.138)</td>
</tr>
<tr>
<td>Myopic choroidal neovascularization</td>
<td>53.6 ± 38.2</td>
<td>52.7 ± 38.5 (1.00)</td>
<td>51.9 ± 38.2 (0.348)</td>
</tr>
</tbody>
</table>

**Notes:** LogMAR, logarithm of the minimum angle of resolution; <sup>a</sup> at the loading phase, all eyes with PCV and AMD received three successive intravitreal injections of ranibizumab at monthly intervals (at baseline and at 1 and 2 months); eyes with myopic CNV were treated by a single injection of ranibizumab; <sup>b</sup> data were analyzed by repeated measures analysis of variance and multiple comparisons were done using the Bonferroni post-hoc method.
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Abbreviation:

Figure 1 Choroidal neovascularization (CNV) associated with age-related macula
degeneration treated with ranibizumab.

Notes: (Top left) Left eye of an 81-year-old man with CNV associated with age-
related macular degeneration. Initial visual acuity in the left eye was 0.2 in a Landolt
chart (0.70 in logarithm of the minimal angle of resolution) and refractive error
was −1.75 Dioptr. (Top middle) Fluorescein angiography shows a minimally classic
CNV. (Top right) Indocyanine green angiography shows no polypoidal lesions.
(Middle) A horizontal section through the fovea obtained by optical coherence
tomography with the enhanced-depth imaging technique before the treatment.
Multiple cystoid spaces are seen. Central retinal thickness was 529 µm, and central
choroidal thickness was 105 µm. (Bottom) A horizontal section through the fovea
after three monthly injections of ranibizumab at the loading phase. Intraretinal
fluid was absorbed completely (central retinal thickness, 157 µm), and visual
acuity improved to 0.3 in a Landolt chart (0.52 in logarithm of the minimal angle of
resolution). The choroidal structure shows no changes (central choroidal thickness,
101 µm). At 9 months, the central choroidal thickness was still 103 µm.
Abbreviation: CSI, choriocapillary interface.

Figure 2 Polypoidal choroidal vasculopathy treated with ranibizumab.

Notes: (Top left) Left eye of a 58-year-old man with polypoidal choroidal
vasculopathy. Initial visual acuity in the left eye was 0.15 in a Landolt chart (0.82 in
logarithm of the minimal angle of resolution). (Top middle) Fluorescein angiography
shows occult with no classic choroidal neovascularization. (Top right) Indocyanine
green angiography shows a branching vascular network terminating in polypoidal
lesions. (Middle) A horizontal section through the fovea obtained by optical
coherence tomography using the enhanced-depth imaging technique before the
treatment shows fibrin exudates with surrounding serous retinal detachment.
Central retinal thickness was 295 µm and central choroidal thickness was 270 µm.
(Bottom) A horizontal section through the fovea after three monthly injections
and an additional injection of ranibizumab. Exudative change was almost absorbed
(central retinal thickness, 235 µm), and visual acuity improved to 0.6 in a Landolt
chart (0.22 in logarithm of the minimal angle of resolution). The choroidal structure
showed no changes, and the central choroidal thickness was 259 µm.
Abbreviation: CSI, choriocapillary interface.

Recently, Maruko et al26 reported that subfoveal choroidal
thickness is decreased after photodynamic therapy in eyes
with PCV. In addition, the authors suggested that the choroi-
dal thickening seen in PCV eyes is associated with increased
choroidal vascular hyperpermeability.26 After photodynamic
therapy, choroidal thickness in eyes with PCV was reduced
not only in the macular area, where the photodynamic therapy
was performed, but also beyond the vascular arcade. Photod-
ynamic therapy could possibly reduce choroidal thickness
by decreasing choroidal vascular hyperpermeability. Our
patients with PCV showed no significant reduction in chorio-
dal thickness after ranibizumab treatments. Although VEGF
works to increase the vascular permeability in the eye,42 the
effect of the ranibizumab on choroidal vascular permeability
may be limited.

Papadopoulou et al43 recently reported a reduction in the
vessel caliber of the retinal arterioles in eyes with AMD after
treatment with ranibizumab. In addition, other investigators
have reported the vasoconstriction44 or even occlusion of
retinal arterioles45,46 induced by intravitreal injections of
bevacizumab, another anti-VEGF agent. Because VEGF
we studied only the change in the thickness of the choroid and did not evaluate changes in its function. It is possible that choroidal blood flow changes with ranibizumab treatment. Therefore, it may be necessary to evaluate choroid function during ranibizumab treatment as well.

**Acknowledgements**

This study was supported in part by the Japan Society for the Promotion of Science (JSPS), Tokyo, Japan (Grant-in-Aid for Scientific Research, no 21592256), and by the Japan National Society for the Prevention of Blindness, Tokyo, Japan.

Contributions of the authors were as follows: Conception and design of the study (AAE, AT, NY); analysis and interpretation (AAE, AT, KO, SO, KY, AO, NY); writing of the article (AAE, AT); critical revision of the article (KO, SO, KY, AO, NY); final approval of the article (AAE, AT, KO, SO, KY, AO); and data collection (AAE, AT, KO, SO, KY, AO).

The Institutional Review Board and Ethics Committee of Kyoto University approved this study, which adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from each subject before examination.

**Figure 4** Changes in mean central retinal thickness and mean central choroidal thickness during ranibizumab treatment for choroidal neovascularization (CNV) in eyes with age-related macular degeneration (top left), polypoidal choroidal vasculopathy (top right), and myopic CNV (second row).

**Notes:** The black squares indicate central retinal thickness; the open circles indicate central choroidal thickness. *P*, 0.01, compared with values before treatment. At the loading phase, all eyes with PCV and AMD received three successive intravitreal injections of ranibizumab at monthly intervals (at baseline and at 1 and 2 months); eyes with myopic CNV were treated by a single injection of ranibizumab.

is reported to induce vascular dilation and increase retinal blood flow,\(^{12}\) pan-inhibition of VEGF by ranibizumab may cause vasoconstriction of retinal arterioles. However, we found no prominent effects of ranibizumab on choroidal thickness; it is unclear why no effects were seen. One possibility is that the concentration of ranibizumab in the choroid is low due to the blockage of the retinal pigment epithelium. Another possibility is that VEGF has a limited role in the control of blood flow and vascular permeability in the choroid.

Major limitations of the current study are its small sample size, lack of controls, and short follow-up period. Another limitation is that measurement of choroidal thickness was performed manually and only at the fovea. The measurement of choroidal thickness tends to be affected by focal irregularity or indistinctness of the choriocapillaris border.\(^{23,24,40}\) The raster scan protocol, which uses many measurement points from an OCT at a longer wavelength, would minimize measurement errors. Software to determine the borders of the choroid automatically is essential to standardize this technique further.\(^{41,47,48}\) Despite these shortcomings, our findings suggest that the effect of ranibizumab on choroidal thickness can be estimated to be minimal, if any. However, we studied only the change in the thickness of the choroid and did not evaluate changes in its function. It is possible that choroidal blood flow changes with ranibizumab treatment. Therefore, it may be necessary to evaluate choroid function during ranibizumab treatment as well.
Disclosure
The authors report no conflicts of interest in this work.

References


