

Pattern Electrophoretography in Age-Related Macular Degeneration

Arsham Sheybani, MD; Milam A. Brantley Jr, MD, PhD; Rajendra S. Apte, MD, PhD

Objective: To determine whether prolonged vascular endothelial growth factor inhibition is toxic to the retina by using pattern electrophoretographic imaging in participants with neovascular age-related macular degeneration (AMD).

Methods: We performed a prospective, single-arm clinical trial of 17 eyes in 17 treatment-naïve participants with subfoveal choroidal neovascularization from AMD. On-label intravitreal ranibizumab was injected monthly for 6 months. Then pattern electrophoretographic imaging was performed before and at 1 month, 3 months, and 6 months after first treatment, and results were interpreted by a trained reader masked to the clinical data. The primary outcome measure was the change in pattern electrophoretographic imaging (positive wave peaking at 50 milliseconds [P50] and negative wave peaking at 95 milliseconds [N95] values) from baseline at 6 months. The secondary outcome measure was the change in visual acuity at 6 months.

Results: The mean participant age was 79.6 years (range, 69.5-90.4 years). At baseline, mean (SD) P50 and N95 amplitudes were 1.3 (0.69) μ V and 1.5 (0.71) μ V, respectively. By 6 months, no decrease in P50 or N95 amplitudes from baseline was observed (1.4 [0.47] μ V, $P = .46$; and 1.8 [0.96] μ V, $P = .14$, respectively). Mean visual acuity before treatment was 20/85 with improvement to a mean of 20/55 ($P = .004$) at 6 months.

Conclusions: This study found no decrease in P50 and N95 amplitudes in participants treated with ranibizumab for neovascular AMD. These findings indicate that vascular endothelial growth factor inhibition with monthly injections of ranibizumab for 6 months likely does not lead to retinal damage.

Trial Registration: clinicaltrials.gov Identifier: NCT00500344

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AGE-RELATED MACULAR DEGENERATION (AMD) is a progressive disease that causes irreversible visual impairment and blindness in nearly 50 million people worldwide.^{1,2} Although geographic atrophy and neovascularization represent the advanced forms of AMD, neovascular AMD is the more aggressive form and accounts for almost 90% of blindness from this disease.³ It is characterized by choroidal neovascularization (CNV), which is the development of abnormal blood vessels underneath the retina. Vascular endothelial growth factor (VEGF) is a prime mechanism of this process.^{4,5}

Targeted therapy against VEGF for neovascular AMD was recently investigated in 2 randomized clinical trials: Minimally Classic/Occult Trial of the Anti-VEGF Antibody Ranibizumab in the Treatment of Neovascular AMD (MARINA) and Anti-VEGF Antibody for the Treatment of Predominantly Classic CHORoidal Neovascularization in AMD (ANCHOR).^{6,7} Both

trials treated participants with ranibizumab (Lucentis; Genentech Inc, South San Francisco, California), a monoclonal antibody that inhibits the isoform VEGF-A, with monthly injections during a 24-month period. The data demonstrate that in patients with subfoveal CNV from AMD, continued intravitreal therapy with ranibizumab leads to stabilization of vision in more than 90% of patients. In addition, at least one-third of patients experienced a significant improvement in vision (≥ 15 letters) by the end of the study period. These results led to US Food and Drug Administration approval of ranibizumab (0.5 mg) for the treatment of neovascular AMD. Although these trials also showed that intraocular injections of ranibizumab were well tolerated locally and systemically, retinal function other than visual acuity (VA) was not assessed.

Recent data have suggested potential toxicity of anti-VEGF-A therapy to retinal ganglion cells because VEGF is an important mediator of retinal neuroprotection by inhibiting apoptosis and promoting

Author Affiliations: Department of Ophthalmology and Visual Sciences, School of Medicine, Washington University in St Louis (Drs Sheybani, Brantley, and Apte), and Barnes Retina Institute (Drs Brantley and Apte), St Louis, Missouri.

cell survival.^{8,9} In an ischemia-reperfusion model of the rat eye, ischemic preconditioning led to higher levels of VEGF-A. This finding correlated with a decrease in the number of retinal cells undergoing apoptosis. With VEGF-A inhibition, the antiapoptotic effects of preconditioning were reversed, suggesting that VEGF-A is critical in this process.¹⁰ Although evidence exists of VEGF-mediated neuroprotection in animal models, minimal clinical data address retinal function after anti-VEGF blockade in ranibizumab-treated human eyes.

Electroretinography (ERG) can be used as a measure of retinal function. Although full-field ERG assesses overall retinal function, pattern ERG (PERG) can record a single response throughout the central visual field.^{11,12} Pattern ERG is a retinal potential evoked when a high-contrast, patterned stimulus is viewed. This is usually performed by alternating black and white checkerboards or stripes, eliciting a positive wave peaking at 50 milliseconds (P50) and a negative wave peaking at 95 milliseconds (N95) after the contrast is reversed. It provides not only a measure of macular function (P50) but also a measurement of ganglion cell function (N95).¹¹ Multifocal ERG also can be used to evaluate focal responses believed to be cone derived. Although multifocal ERG can be used to assess central retinal function, it does not provide a specific measure of ganglion cell function.¹³ However, PERG directly quantifies ganglion cell function.¹¹ Thus, PERG provides a quantifiable means of assessing macular and ganglion cell function in the context of anti-VEGF therapy. In this study, treatment-naïve patients with subfoveal neovascular AMD were treated per label monthly for 6 months with intravitreal ranibizumab and their macular function was evaluated via PERG.

METHODS

Ours was a prospective, single-arm study of intravitreal ranibizumab administered on-label in treatment-naïve patients followed up by serial pattern electroretinographic imaging. The institutional review board and Human Research Protection Office at Washington University in St Louis, as well as the study office at Barnes Retina Institute, approved the study.

Inclusion and exclusion criteria are presented in **Table 1**. Consented, enrolled study participants received intravitreal injections of 0.5 mg of ranibizumab administered monthly for 6 months. Then pattern electroretinographic imaging was performed at baseline, 1 month, 3 months, and 6 months and graded by a masked trained reader (M.A.B.). The primary outcome measure was the change in pattern electroretinographic imaging (P50 and N95 values) from baseline to 6 months. The secondary outcome measure was the change in VA at 6 months from baseline.

Baseline VA measurement, time-domain optical coherence tomography (OCT), and fluorescein angiography were performed for each patient at the initial visit, as depicted in **Table 2**. The VAs were recorded as Snellen equivalents after an Early Treatment of Diabetic Retinopathy Study standardized refraction. The optical coherence tomographic images and fluorescein angiograms were interpreted by the treating physician. The OCT was performed at the final visit, 1 month after the sixth intravitreal injection. Resolution (or lack thereof) of fluid on optical coherence tomographic imaging was interpreted by the treating physician.

The checkerboard transient PERG was recorded with the Espion E² Electrophysiology System (Diagnosys LLC, Boston,

Table 1. Inclusion and Exclusion Criteria

Type	Criteria
Inclusion criteria	Age ≥50 y Treatment-naïve AMD patients determined by the investigators to be candidates for ranibizumab therapy Visual acuity 20/40 to 20/320
Exclusion criteria	Previous therapy for AMD or other retinal disease that may be used in the treatment of AMD Any other condition the investigator believes would pose a significant hazard to the patient if on-label ranibizumab were prescribed Any condition that would interfere with the electroretinographic recording (eg, media opacities, including lens or corneal opacity) Concurrent eye disease in the study eye that could compromise visual acuity (eg, severe diabetic retinopathy or advanced glaucoma) Any condition causing the patient to have a significant tremor that would interfere with his or her ability to remain motionless during the electroretinographic recording (eg, severe Parkinson disease) Participation in another simultaneous medical investigation or trial

Abbreviation: AMD, age-related macular degeneration.

Massachusetts) in accordance with the guidelines of the International Society for Clinical Electrophysiology of Vision.¹⁴ The PERGs were recorded using Dawson, Trick, and Litzkow fiber electrodes (Diagnosys LLC) moistened with 1% carboxymethylcellulose sodium placed in the inferior fornix of each eye. Each Dawson, Trick, and Litzkow (DTL) fiber was laid along the top of the lower eyelids so that it contacted the inferior cornea. Fibers were anchored with small adhesive pads near the inner and outer canthi. Reference electrodes were applied with adhesive paste lateral to the outer canthi. An adhesive electrode placed on the forehead served as the ground. The distance between the eye of the patient and the monitor was 30 cm when using the standardized Early Treatment of Diabetic Retinopathy Study refraction. The responses on pattern electroretinographic imaging were recorded, including P50 (derived from macular photoreceptors) and N95 (postphotoreceptor, including the optic nerve), in response to an alternating checkerboard pattern. During recordings, bandpass filter amplifiers included the range from 1 to 100 Hz. The pattern electroretinographic image was obtained using a mean (SD) reversal rate of 4 (0.8) revolutions per second, and the mean of the width and height of the stimulus field was 15° with a check size of 0.8°. The P50 amplitude was measured from the trough of N35 to the peak of P50. The N95 amplitude was measured from the peak of P50 to the trough of N95.

Statistical analyses were performed using the paired *t* test to analyze VA and pattern electroretinographic variables. *P* ≤ .05 was considered statistically significant. Results were expressed as mean (SD). For analysis, VA was converted to logMAR equivalents and represented as mean VA in Snellen format.

RESULTS

Seventeen patients from the Barnes Retina Institute were enrolled in the study. All patients completed the study per the study design. The mean patient age was 79.6 years (range, 69.5-90.4 years). Most patients were women (14 [82%]), with a roughly equal distribution of CNV lesion types: 8 predominantly classic (47%) and 9 occult

Table 2. Study Design

Variable	Visit 1 (Day 0)	Visit 2 (Month 1)	Visit 3 (Month 2)	Visit 4 (Month 3)	Visit 5 (Month 4)	Visit 6 (Month 5)	Visit 7 (Month 6)
ETDRS visual acuity score	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Pattern electroretinography	Yes	Yes	...	Yes	...	...	Yes
Fluorescein angiography	Yes	...	...	...	...	...	...
Intravitreal injection	Yes	Yes	Yes	Yes	Yes	Yes	...
Intraocular pressure	Yes	Yes	Yes	Yes	Yes	Yes	...
Initial examination and medical history	Yes	...	...	...	...	...	...
Demographic data	Yes	...	...	...	...	...	...
Informed consent	Yes	...	...	...	...	...	...
Optical coherence tomography	Yes	...	...	...	...	...	Yes

Abbreviations: ellipses, not applicable; ETDRS, Early Treatment of Diabetic Retinopathy Study.

Table 3. Baseline Patient Characteristics and Clinical Treatment Outcomes

Variable	Patients (N=17) ^a
Age, mean (range), y	79.6 (69.5-90.4)
Sex	
Male	3 (18)
Female	14 (82)
Eye	
Right	8 (47)
Left	9 (53)
Prior cataract extraction	6 (35)
CNV in study eye	
Classic	8 (47)
Occult	9 (53)
CNV in nonstudy eye	8 (47)
Ranibizumab treatment in nonstudy eye	7 (41)
Fluid resolution at end of study per optical tomographic imaging results	13 (76)
Preinjection VA (Snellen equivalent)	20/85 ^b
Postinjection VA (Snellen equivalent)	20/55 ^b

Abbreviations: CNV, choroidal neovascularization; VA, visual acuity.

^aData are presented as number (percentage) of patients unless otherwise indicated.

^b $P = .004$.

(53%) (with or without a classic component) (**Table 3**). By the end of the study period, fluid resolution as determined via OCT was observed in 13 patients (76%).

The results of pattern electroretinographic imaging were deemed uninterpretable for 1 patient and were not incorporated into the statistical analysis. The tracings showed little statistical decrease in P50 or N95 amplitudes during the 6-month period (**Table 4**). At baseline, mean P50 and N95 amplitudes were 1.3 (0.69) μ V and 1.5 (0.71) μ V, respectively. By 3 months, P50 tracings showed improvement from preinjection values (1.7 [0.85] μ V, $P = .049$) with no significant change in N95 amplitudes (1.8 [1.04] μ V). The P50 gains were not sustained by 6 months after initial treatment. At the end of the study period, no decrease was observed in P50 or N95 amplitudes from baseline (1.4 [0.47] μ V, $P = .46$; and 1.8 [0.96] μ V, $P = .14$, respectively). Representative tracings are shown in the **Figure**. The secondary outcome showed statistically significant improvement from baseline because mean VA before treatment was 20/85 (range, 20/32-20/400) with improvement by the end of the study

period to a mean of 20/55 (range, 20/20-20/400) ($P = .004$). No difference was observed in amplitudes between classic and occult lesions.

COMMENT

Intravitreal ranibizumab has gained widespread use for the treatment of neovascular AMD. Animal studies implicate VEGF as critical in neuroprotection and in the context of the large number of patients receiving anti-VEGF therapy; a study to assess retinal function would be timely. Recent work in animal models also confirms that a significant role for VEGF exists in the prevention of apoptosis of retinal Müller cells and photoreceptors.¹⁵ This work shows that systemic VEGF neutralization (for as little as 2 weeks) in mice led to Müller cell apoptosis with a decline of retinal function as assessed by ERG. To our knowledge, no conclusive answer exists regarding whether long-term VEGF inhibition in humans with neovascular AMD may lead to retinal cell damage. As reported in the clinical trials cited in this article, central vision in neovascular AMD improves with anti-VEGF therapy; however, this only signifies improvement in less than 1° of the central retina. By comparison, PERG assesses the central 15° and also quantifies ganglion cell function in this area.

To date, studies addressing retinal function after treatment with anti-VEGF therapies in human eyes are limited. Currently, 2 anti-VEGF antibodies are in clinical use (bevacizumab and ranibizumab), and both neutralize an isoform of VEGF (VEGF-A). The full-length anti-VEGF antibody bevacizumab has been prospectively evaluated for retinal toxicity by pattern electroretinographic imaging in a few studies. One study¹⁶ evaluated 35 patients with diabetic macular edema treated with bevacizumab only. No evidence was observed of macular or ganglion cell damage as determined via pattern electroretinographic imaging. This study even showed a significant improvement in P50 and N95 amplitudes during 3 months. These patients were not treated according to a standard protocol, receiving a variable number of injections dependent on the presence of macular edema for only 3 months. Another prospective study¹⁷ evaluated PERG in patients with neovascular AMD treated with photodynamic therapy with verteporfin only or combination photodynamic therapy and intravitreal bevacizumab. The results revealed no evidence of retinal

Table 4. Pretreatment and Posttreatment P50 and N95 Values

Amplitude	Pretreatment	Months			P Value ^a
		1	3	6	
P50, μ V	1.3 (0.69)	1.5 (0.63)	1.7 (0.85)	1.4 (0.47)	.46
N95, μ V	1.5 (0.71)	1.7 (0.90)	1.8 (1.04)	1.8 (0.96)	.14

Abbreviations: N95, negative wave peaking at 95 milliseconds; P50, positive wave peaking at 50 milliseconds.

^aCalculated from pretreatment period to the 6-month time point.

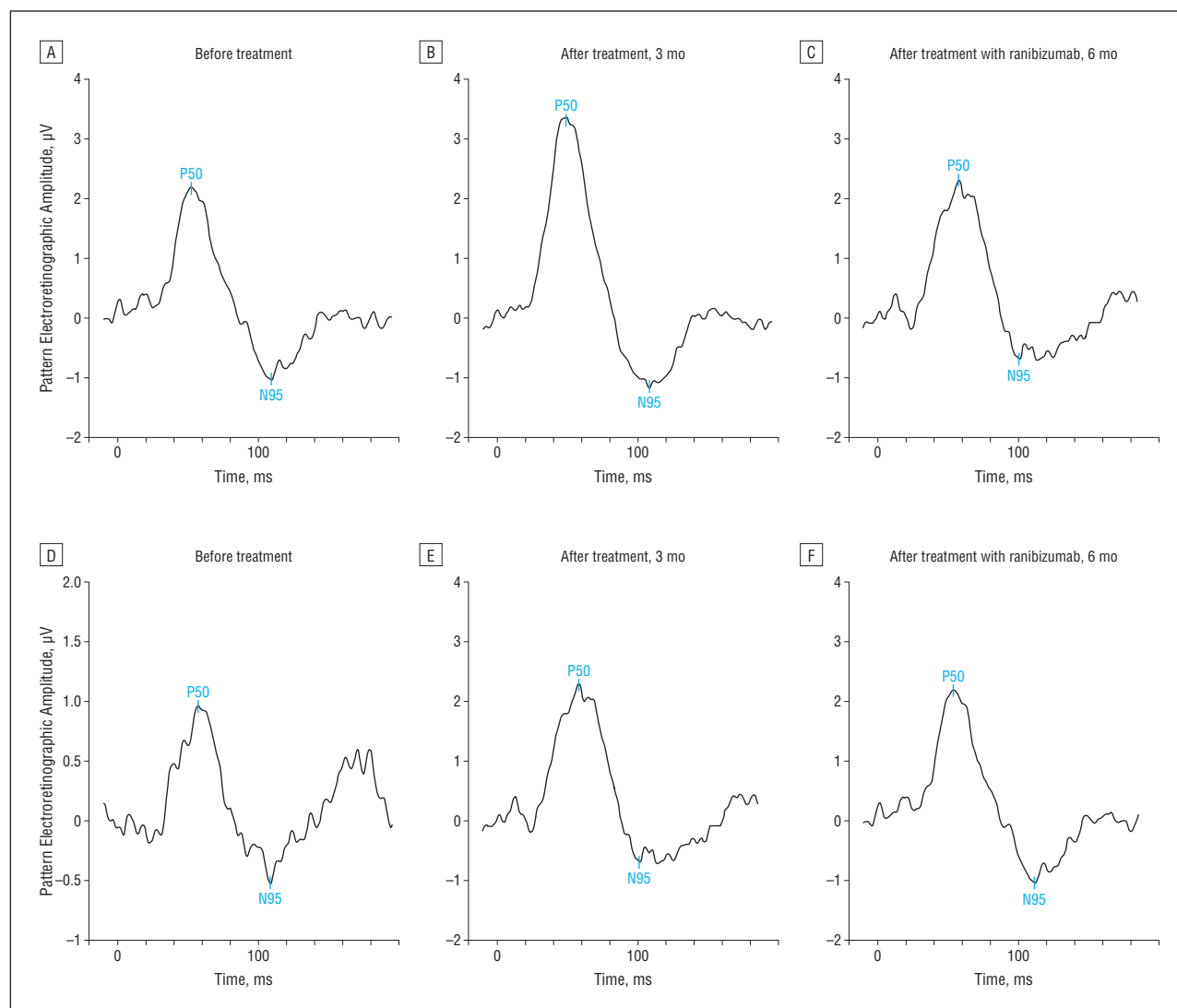


Figure. Pattern electroretinographic recordings from 2 patients (patient 1 [top row] and patient 2 [bottom row]). A and D, Before treatment; B and E, 3 months after treatment with ranibizumab; and C and F, 6 months after treatment with ranibizumab. N95 indicates negative wave peaking at 95 milliseconds; P50, positive wave peaking at 50 milliseconds.

damage as determined by pattern electroretinographic imaging with significant improvement in P50 amplitudes from baseline in both treatment groups. This study was limited by the short follow-up time of only 1 month.

Retinal function data for ranibizumab also are limited. Lücke and colleagues¹⁸ studied ranibizumab and its effects on retinal function with an ex vivo model of bovine retinas. Postmortem bovine eyes were superfused

with supratherapeutic concentrations of ranibizumab (equivalent to a 1-mg injection), and subsequent electroretinographic recordings were performed. After a 45-minute perfusion of ranibizumab, the a-wave amplitude was only reduced by 4% with no statistical difference from baseline. Although this finding provides interesting ex vivo animal data, it is important to record retinal function in vivo by monitoring retinal function in patients receiving multiple injections for an extended period.

A prospective study¹⁹ of 3 patients with AMD evaluated retinal function after 3 ranibizumab (0.3 mg) injections. Only 2 of the patients were treatment naive. One patient had received photodynamic therapy 3 times before ranibizumab for recurrent CNV. These 3 patients were compared to 20 healthy patients of similar age, and multifocal ERG was performed after each of the 3 treatments. This study found that when compared with control individuals, central and peripheral N1P1 amplitudes were significantly less after 3 treatments with ranibizumab. However, when compared with the pretreatment levels of treated patients, no significant decrease was observed in N1P1 amplitudes. It could not be concluded whether the decreased amplitude compared with controls was due to the natural progression of the disease or possibly secondary to retinal toxic effects after treatment. The study was limited by a small sample size, the lack of all patients being treatment naive, a ranibizumab dose of 0.3 mg, and a relatively short follow-up period. The study authors suggested the need for a prospective trial to further explore their initial findings.

Our study found no evidence of ganglion cell damage secondary to ranibizumab in neovascular AMD as measured by PERG. The VA outcomes in our study also mirrored prior prospective clinical trial data.^{6,7} Given the prospective design in treatment-naïve patients and the masked nature of the analysis, this study provides evidence of the preservation of macular and retinal ganglion cell function after long-term VEGF inhibition in patients with neovascular AMD.

We acknowledge that the study was limited by its small sample size and lack of a control group. The strength of this study lies in the prospective design with treatment-naïve patients, which provides valuable data regarding the safety of intravitreal ranibizumab in regard to retinal ganglion cell function. These findings indicate that repeated VEGF inhibition in our cohort likely does not lead to macular and ganglion cell damage as assessed by PERG.

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Correspondence: Rajendra S. Apte, MD, PhD, Department of Ophthalmology and Visual Sciences, School of Medicine, Washington University in St Louis, 660 S Euclid Ave, PO Box 8096, St Louis, MO 63110 (apte@vision.wustl.edu).

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