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Changes in Retinal Nonperfusion Associated with Suppression of Vascular Endothelial Growth Factor in Retinal Vein Occlusion

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Purpose: To assess changes in retinal nonperfusion (RNP) in patients with retinal vein occlusion (RVO) treated with ranibizumab.

Design: Secondary outcome measure in randomized double-masked controlled clinical trial.

Participants: Thirty-nine patients with central RVO (CRVO) and 42 with branch RVO (BRVO).

Methods: Subjects were randomized to 0.5 or 2.0 mg ranibizumab every month for 6 months and then were re-randomized to pro re nata (PRN) groups receiving either ranibizumab plus scatter laser photocoagulation or ranibizumab alone for an additional 30 months.

Main Outcome Measures: Comparison of percentage of patients with increased or decreased area of RNP in patients with RVO treated with 0.5 versus 2.0 mg ranibizumab, during monthly injections versus ranibizumab PRN, and in patients treated with ranibizumab PRN versus ranibizumab PRN plus laser.

Results: In RVO patients given monthly injections of 0.5 or 2.0 mg ranibizumab for 6 months, there was no significant difference in the percentage who showed reduction or increase in the area of RNP. However, regardless of dose, during the 6-month period of monthly injections, a higher percentage of patients showed a reduction in area of RNP and a lower percentage showed an increase in area of RNP compared with subsequent periods of ranibizumab PRN treatment. After the 6-month period of monthly injections, BRVO patients, but not CRVO patients, randomized to ranibizumab PRN plus laser showed significantly less progression of RNP compared with patients treated with ranibizumab PRN.

Conclusions: Regardless of dose (0.5 or 2.0 mg), monthly ranibizumab injections promote improvement and reduce progression of RNP compared with PRN injections. The addition of scatter photocoagulation to ranibizumab PRN may reduce progression of RNP in patients with BRVO, but a statistically significant reduction was not seen in patients with CRVO. *Ophthalmology 2016;123:625-634 © 2016 by the American Academy of Ophthalmology.*

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Retinal vein occlusion (RVO) is a prevalent retinal vascular disease that is subdivided into central RVO (CRVO), in which there is occlusion of the main outflow vessel of the eye, and branch RVO (BRVO), in which a branch of the central retinal vein is occluded. They differ in the amount of retina affected by the occlusion, and on average, CRVO tends to have a worse visual prognosis than BRVO. There is considerable overlap in molecular pathogenesis because in both, retina drained by occluded vessels becomes ischemic and produces hypoxia-regulated gene products, including vascular endothelial growth factor (VEGF). A pilot trial indicated that VEGF is a major contributor to macular edema, because suppression of VEGF by intraocular injections of ranibizumab reduced edema and improved visual acuity.[1] This was confirmed in large multicenter phase 3 trials.[2,3] Injections of another VEGF antagonist, aflibercept, have shown similar effects.[4]

Studies with ranibizumab have uncovered additional deleterious effects of high intraocular levels of VEGF that are reversed by ranibizumab. Patients with RVO treated with monthly injections of ranibizumab show more rapid resolution of retinal hemorrhages, indicating that VEGF promotes ongoing hemorrhaging that is blocked by ranibizumab.[5,6] Measurement of the area of retinal nonperfusion (RNP) in the macula by masked grading of fluorescein angiography (FA) images at an independent reading center demonstrated progression of central RNP in sham-treated patients with RVO that was reduced significantly in patients given monthly injections of ranibizumab for 6 months.[7] Some patients in the ranibizumab treatment group

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showed reduction in RNP in the macula over the first 6 months. After 6 months, ranibizumab injections were given to patients who previously received sham injections, and the differences from baseline RNP between the groups was eliminated. This suggests that high levels of VEGF promote closure of retinal vessels and that neutralization of VEGF can prevent additional vessel closure and can even cause recently closed vessels to reopen. This is a revolutionary concept, and as is usually the case with new and unexpected findings, it is difficult for many clinicians and researchers to accept. One possible concern is that 30° FA images were used to visualize and grade RNP, and therefore only the macula and surrounding area of the retina was assessed. There is no reason to believe that vessels in the posterior retina should differ from those in the peripheral retina in their response to high levels of VEGF, but it would be useful to demonstrate this.

After initiation of the Ranibizumab Dose Comparison (0.5 and 2.0 mg) and the Role of Laser in the Management of Retinal Vein Occlusion (RELATE) trial,[8] the study protocol was amended to include as a secondary end point, the effect of VEGF neutralization on RNP of peripheral as well as central retinal vessels using ultra−wide-field FA. The following experimental questions were addressed. First, during a 6-month period of monthly injections of ranibizumab, compared with RVO patients treated with 0.5 mg ranibizumab, do a higher percentage of patients treated with 2.0 mg ranibizumab show a reduction in area of RNP, do a lower percentage show an increase in area of RNP, or both? Second, during a period when RVO patients are given an injection of ranibizumab every month regardless of dose, is there a higher percentage with reduction in area of RNP, a lower percentage with increase in area of RNP, or both compared with a period when the same patients are given pro re nata (PRN) injections? Third, compared with patients with RVO treated with ranibizumab PRN, do patients treated with ranibizumab PRN plus laser show a higher percentage with reduction in the area of RNP, a lower percentage with an increase in area of RNP, or both? The results are reported herein.

Methods

The RELATE trial (ClinicalTrials.gov identifier, NCT01003106) was an investigator-initiated double-masked randomized trial sponsored by Genentech, Inc. (South San Francisco, CA) and designed to compare the effects of monthly injections of 0.5 mg ranibizumab with monthly injections of 2.0 mg ranibizumab for 6 months in patients with macular edema resulting from RVO and to also determine if scatter−grid laser photocoagulation reduces the need for injections and improves long-term outcomes. The study was conducted in accordance with the Declaration of Helsinki, applicable United States Food and Drug Administration regulations, and the Health Insurance Portability and Accountability Act. The study protocol was approved by the Johns Hopkins University Institutional Review Board before study initiation, and all participating patients provided informed consent. The design and details of the treatment protocol of the RELATE trial have been published previously and are not repeated here.[8] After initiation of the trial, ultra−wide-field FA images using the Optos 200Tx imaging system (Optos PLC, Dunferlmine, UK) were provided to evaluate the effect of VEGF suppression on retinal vessel perfusion in the periphery by providing a 200° view of the retina in a single photograph. Some patients had already entered the trial, and in those patients, 7 field images with a 30° fundus camera had been obtained for baseline FA images, but all subsequent FA images in those patients were obtained with an Optos 200Tx imaging system, and all patients enrolled after that point underwent ultra−wide-field FA imaging at baseline and months 6, 12, 24, and 36.

Nonperfusion was evaluated throughout the entire retina, including both the posterior and peripheral retina, on ultra−wide-field FA images. Experienced graders were masked with respect to randomization groups. The ultra−wide-field FA images obtained at each time point starting with month 6 were compared with those obtained at the previous time point. Although it is clearly advantageous to assess a larger area of the retina, one disadvantage of the ultra−wide-angle images is that many of the images have blurring and artifact in the far periphery and the amount of blurring varies from patient to patient; some patients are able to keep their head in the right position and their eyes wide open and others have difficulty doing so, resulting in more edge artifact. Thus, the amount of retina that can be assessed varies from patient to patient, and measurement of amount of RNP cannot be compared between patients. However, it is feasible to make longitudinal assessments in the same patient and to determine if the amount of RNP at one time point is increased, decreased, or the same at the next time point for the same area of retina assessed. It is for this reason that categorical grading was performed rather than attempting to provide an absolute amount of RNP in each image, because the latter implies a level of precision that is not possible and would be misleading. In addition, our experimental questions required categorical grading and not absolute measurements. To make a change assessment between 2 time points, it is was necessary to have FA images at each time point that were sufficiently clear with only small areas of edge artifact allowing the grading to be made with a high level of confidence. In general, if there was more than a 10% difference in retinal vascular area that could be graded between images, they were judged ungradable. Also, if a region that showed RNP on one image was obscured on the other image because of edge artifact and the region was sufficiently large so that a difference in that region could affect the grading, the images were listed as ungradable.

All statistical tests were performed using IBM SPSS software version 19.0 (IBM Corp., Armonk, NY) and Stata software version 13.1 (College Station, TX). For categorical variables, comparisons between groups were made using the chi-square test for a large sample size and the Fisher exact test for a small sample size. Association between variables was sought using the Spearman's rank correlation coefficient (ρ). Independent analysis was run for each variable at month 6, 12, 24, and 36. To compare the change in area of RNP on consecutive FA images, multinomial logistic regression models with robust standard error estimation (Huber-White-sandwich estimator of the variance) were used to account for the correlation among the repeated measures from the same patients.[9] For patients who missed 1 or more of the end point visits, ultra−wide-field FA images obtained within 3 months of the end point visit were used for grading.

Results

In the RELATE trial, 81 patients with RVO (39 with CRVO and 42 with BRVO) were enrolled at a single center (Wilmer Eye Institute, Johns Hopkins Hospital, Baltimore, MD) and were randomized to receive injections of 0.5 or 2.0 mg ranibizumab every month with