Vascular Endothelial Growth Factor Is a Critical Stimulus for Diabetic Macular Edema

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• PURPOSE: The role of vascular endothelial growth factor (VEGF) in diabetic macular edema (DME) was tested with ranibizumab, a specific antagonist of VEGF.
• DESIGN: A nonrandomized clinical trial.
• METHODS: Ten patients with chronic DME received intraocular injections of 0.5 mg of ranibizumab at baseline and at one, two, four, and six months. The primary outcome was change in foveal thickness between baseline and seven months, and the secondary outcome measures were changes from baseline in visual acuity and macular volume.
• RESULTS: Mean values at baseline were 503 μm for foveal thickness, 9.22 mm^3 for macular volume, and 28.1 letters (20/80) read on an Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity chart. At seven months (one month after the fifth injection), the mean foveal thickness was 257 μm, which was a reduction of 246 μm (85% of the excess foveal thickness present at baseline; $P = .005$ by Wilcoxon signed-rank test for likelihood that this change is due to ranibizumab rather than chance). The macular volume was 7.47 mm^3, which was a reduction of 1.75 mm^3 (77% of the excess macular volume at baseline; $P = .009$). Mean visual acuity was 40.4 letters (20/40), which was an improvement of 12.3 letters ($P = .005$). The injections were well-tolerated with no ocular or systemic adverse events.
• CONCLUSION: Intraocular injections of ranibizumab significantly reduced foveal thickness and improved visual acuity in 10 patients with DME, which demonstrated that VEGF is an important therapeutic target for DME. A randomized, controlled, double-masked trial is needed to test whether intraocular injections of ranibizumab provide long-term benefit to patients with DME. (Am J Ophthalmol 2006;142:961–969. © 2006 by Elsevier Inc. All rights reserved.)

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DIABETIC RETINOPATHY IS THE MOST PREVALENT cause of vision loss in working aged individuals in developed countries.[1] Severe vision loss occurs because of traction retinal detachments that complicate retinal neovascularization, but the most common cause of moderate vision loss is macular edema. Macular edema occurs from the leakage of plasma into the central retina, which causes it to thicken because of excess interstitial fluid. The excess interstitial fluid is likely to disrupt ion fluxes and the thickening of the macula results in stretching and distortion of neurons. There is reversible reduction in visual acuity, but over time the perturbed neurons die, which results in permanent visual loss.

The leakage of plasma in patients with diabetic macular edema (DME) is visualized by fluorescein angiography and may be focal because of leakage from microaneurysms or diffuse. Microaneurysms are thought to occur because of hyperglycemia-induced pericyte death, which weakens the walls of retinal vessels and results in the small aneurysms in which endothelial cells are perturbed causing them to lose their barrier qualities and leak.[2] However, diffuse leakage from retinal capillaries that do not show visible structural changes (such as microaneurysms) is also a common feature of DME. This could be due to microscopic damage to retinal vessels that are not visible in images that are obtained during fluorescein angiography but could also be due the presence of excessive amounts of pro-permeability factors.

Recently, retinal hypoxia has been implicated in the pathogenesis of DME.[3] Hypoxia causes increased expression of vascular endothelial growth factor (VEGF), which is a potent inducer of vascular permeability that has been shown to cause leakage from retinal vessels.[4,5] Thus, it is

Ranibizumab is a Fab fragment of an antibody that specifically binds all isoforms of VEGF-A with high affinity. Intraocular injections of ranibizumab provide benefit for patients with choroidal neovascularization because of age-related macular degeneration, which confirms studies in animal models that suggest that VEGF is an important stimulus for choroidal neovascularization (reported at the meeting of the American Society of Retina Specialists, Montreal, Canada, July 2005). In this study, we tested the hypothesis that VEGF is also an important stimulus for DME by assessing the effect of multiple intraocular injections of ranibizumab in patients with DME.

METHODS

AN OPEN-LABEL STUDY TO INVESTIGATE THE EFFECT OF intraocular injections of 0.5 mg of ranibizumab in 10 patients with DME was approved by the Federal Drug Administration and the institutional review board of the Johns Hopkins Medical Institutions. The study was designed to give patients an intraocular injection of 0.5 mg of ranibizumab at study entry and at one, two, four, and six months after entry. The dose was selected because 0.5 mg is the highest dose available and because it is reasonable to start with the highest dose and investigate other doses in future studies, if indicated. The regimen was selected to assess the effect of three monthly injections and then to determine the impact of increasing the time between injections to two months for the last two injections. The primary outcome measure was foveal thickness that was measured by optical coherence tomography (OCT)[6,7] at seven months, compared with baseline. Secondary outcome measures were macular volume that was measured by OCT and visual acuity that was measured by the protocol of the Early Treatment Diabetic Retinopathy Study (ETDRS)[8] at seven months, compared with baseline.

- **PATIENT ELIGIBILITY AND EXCLUSION CRITERIA:** Patients (18 or older) were eligible if they had reduction in visual acuity between 20/40 and 20/320 and met the following criteria: (1) baseline foveal thickness by OCT was 250 μm or greater, (2) serum HbA_{1c} ≥6% for 12 months before randomization, (3) no potential contributing causes to reduced visual acuity other than DME, and (4) reasonable expectation that laser photocoagulation would not be required for the next six months. If both eyes were eligible, the eye with the greater foveal thickness was entered.

- **STUDY PROTOCOL:** Consenting patients were screened for the study with a medical history, physical examination, measurement of best-corrected visual acuity by an experienced examiner who used the ETDRS protocol,[8] a complete eye examination, an OCT, a fluorescein angiogram, received an intraocular injection of 0.5 mg of ranibizumab. Patients returned one week later for a repeat examination and OCT. Subsequent return visits occurred every month through seven months, which was the primary end point of the study. Additional injections of ranibizumab were performed at one, two, four, and six months. This protocol was selected to determine the effect of monthly injections for the first three months and then to try to determine whether less frequent injections would be feasible. Safety evaluations, measurement of best-corrected visual acuity, eye examinations, and OCTs were done at all study visits; fluorescein angiograms were done at three and six months. Measurements of HbA_{1C} were done at baseline and three and six months. Hematologic and blood chemistry tests were done at baseline and six months.

- **ADMINISTRATION OF STUDY DRUG:** Povidone iodine was used to clean the lids, and a lid speculum was inserted. Topical anesthesia was applied; in some patients, a subconjunctival injection of 2% lidocaine was given. The conjunctiva was irrigated with 5% povidone iodine. A 30-gauge needle was inserted through the pars plana, and 0.05 ml containing 0.5 mg of ranibizumab was injected into the vitreous cavity. Funduscopic examination was performed to confirm retinal perfusion, and patients were observed for one hour or until intraocular pressure returned to normal. Patients were called the day after each injection and asked whether they had decreased vision, eye pain, unusual redness, or any new symptoms.

- **OCT:** OCT scans were performed by an experienced investigator with a StratusOCT3 (Carl Zeiss Meditec, Dublin, California, USA) that used the fast macular scan protocol. This protocol consists of 6 line scans that are 6.0-mm long, centered on fixation, and spaced 30 degrees apart around the circumference of a circle. Each line consists of 128 A-scan measurements. With each A-scan, the OCT software measures the distance between the inner surface of the retina and the anterior border of the retinal pigment epithelium choriocapillaris complex on the basis of changes in reflectivity. The center point thickness, also known as the foveolar thickness, is a mean value that is generated by the StratusOCT software from the 6 central A-scan thickness values of each of the radial lines comprising the fast macular thickness map. We did not use this value generated from only 6 data points for our primary measure of central retinal thickness but instead used the foveal or central 1 mm thickness, which is an average generated value based on central 21 scans of each of the 6 lines that pass through the patient's fixation. The number of data points that are used to compute this value is $21 \times 6 = 126$, which provides a better representation of the thickness of the central retina than a value that is generated from only 6 points around fixation. Macular volume throughout the entire 6-mm zone is calculated

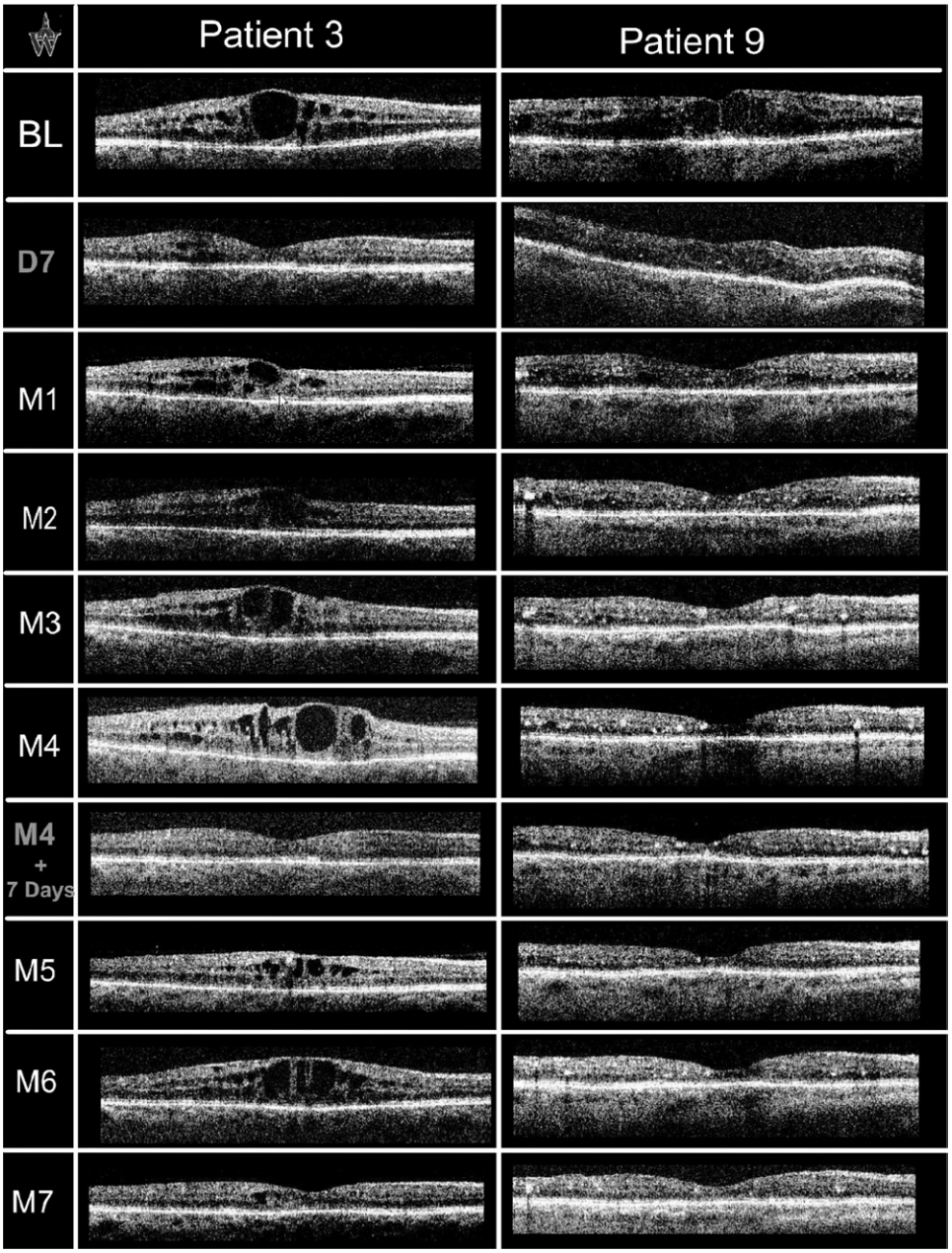

FIGURE 1. Horizontal cross sectional optical coherence tomography (OCT) scans at all time points for patients 3 and 9 with diabetic macular edema that was treated with ranibizumab to illustrate two patterns of response over time. Seven days after the first intraocular injection of 0.5 mg of ranibizumab (day seven), patient 3 showed a marked improvement in the appearance of the OCT scan with the elimination of several large cysts and the return of a normal macular contour that included a foveal depression. At month one (M1), one month after the first injection, and M2 and M3, one month after the second and third injections, respectively, the scans for patient 3 were worse than the scan at day seven, which suggests a loss of effect of ranibizumab or transient effects that are lost by one month after injection. At M4, two months after the third injection, the scan showed substantial deterioration, but seven days after the fourth injection (M4 + seven days) there was marked improvement supporting transient effect. However, there was less deterioration one month after the fourth injection (M5) than there had been one month after each of the first three injections. This was followed by deterioration at M6, two months after the fourth injection, but then at M7, the primary end point and one month after the fifth injection, there was improvement to the point that the scan looked more like the two previous scans that had been performed seven days after an injection than like those scans that had been performed one month after an injection. Like patient 3, patient 9 also showed substantial improvement at day seven compared with baseline, with resolution of several large cysts. However, unlike patient 3, patient 9 showed continued improvement and then stability at subsequent time points, regardless of the time after the injection that the scan was performed. This suggests that the beneficial effects of ranibizumab were more sustained in patient 9 than in patient 3. BL = baseline.

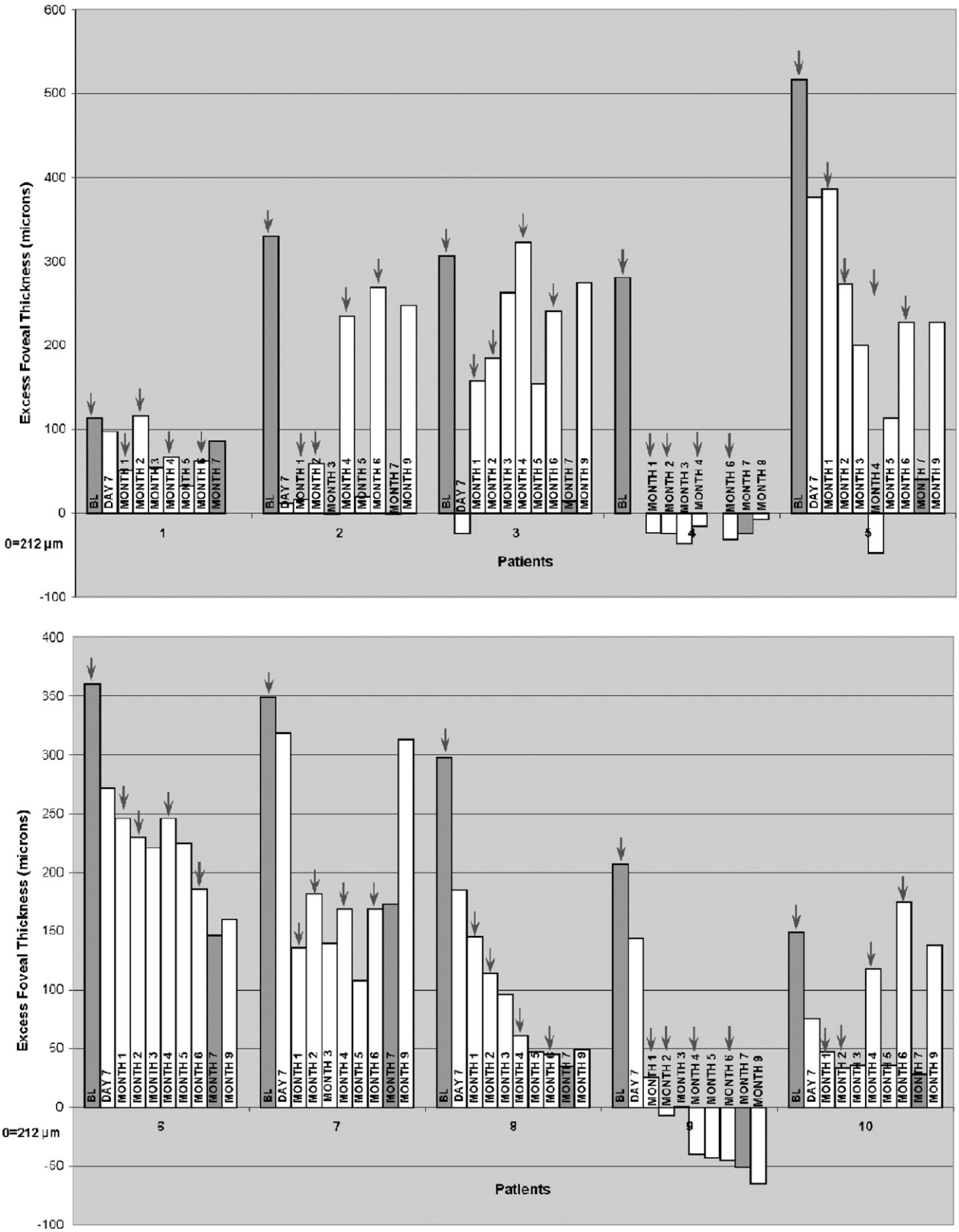

FIGURE 2. Excess foveal thickness was measured by optical coherence tomography (OCT) at each study visit in all patients with diabetic macular edema that was treated with ranibizumab. Each bar represents the foveal thickness above the normal mean value of 212 µm, which is set to zero. The arrows show intraocular injections of 0.5 mg of ranibizumab. The bars for baseline and month seven are shaded to allow quick comparison between baseline and the primary end point. The foveal thickness is less at the primary end point than at baseline for all patients.

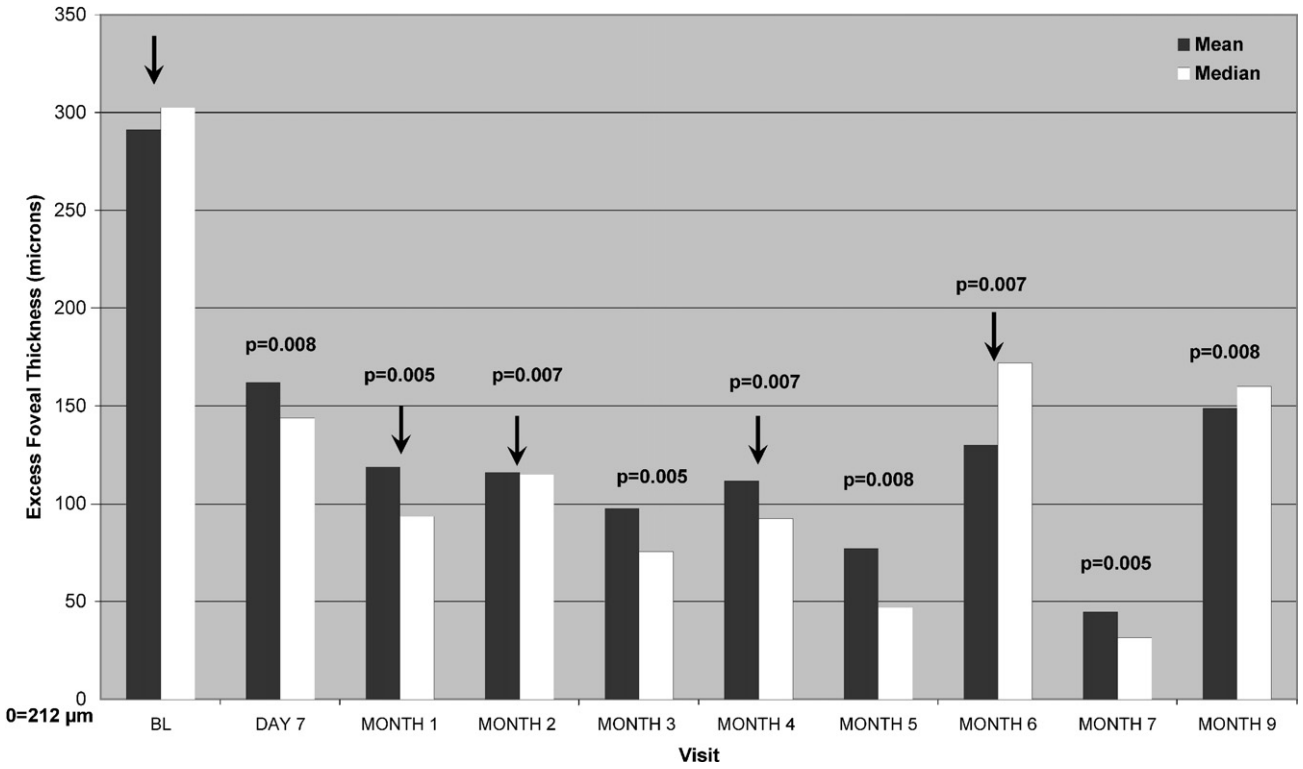

FIGURE 3. The mean excess foveal thickness at each study visit in all patients with diabetic macular edema that was treated with ranibizumab. Each bar represents the mean value for excess foveal thickness for all patients at the designated study visit (data for eight of 10 patients at month nine). The arrows show when intraocular injections of 0.5 mg of ranibizumab were administered. Compared with baseline, foveal thickness was reduced by 246 µm at the primary end point of the study, which constituted the elimination of 85% of the excess foveal thickness that had been present at baseline.

foveal thickness was calculated by subtraction of the measured foveal thickness value from the normal mean value of 212 µm that was calculated from measurements on a large population of subjects.[9] Excess macular volume was determined by subtraction of the upper limit of the normal range of 6.94 ± 0.37 mm^3 from the measured value.

• **STATISTICAL ANALYSIS:** Statistical analyses were performed with Statistical Package for the Social Sciences software (SPSS Inc, Chicago, Illinois, USA). The likelihood that the change in foveal thickness, macular volume, and visual acuity from baseline to month seven was due to ranibizumab rather than to chance was determined by the Wilcoxon signed-rank test.

RESULTS

• **CHARACTERISTICS OF THE STUDY POPULATION:** There were five men and five women in the study, with a median age of 60 years. Eight of the 10 patients were insulin-dependent diabetics. The median and mean HbA_{1C} values at enrollment were 7.50% and 7.64%, respectively, and .240). Four patients had diabetic neuropathy, and three patients had diabetic nephropathy with modest renal insufficiency that did not require dialysis. Eight patients were receiving treatment for hypertension, which was well-controlled; seven patients had hypercholesterolemia, five of whom were receiving treatment. There was no significant change in mean systolic or diastolic blood pressure during the study. The mean duration of DME was 4.75 ± 1.22 years with a median duration of 3.5 years and a range of six months to 10 years. Nine of the 10 patients with DME had received previous treatment in the study eye; eight of the patients had received at least two sessions of focal/grid laser photocoagulation not less than 5 months before study entry (range, five to 120 months), and three patients had received intraocular corticosteroids not less than 10 months before entry (range, 10 to 20 months). Despite these treatments, the mean foveal thickness at baseline was 503 ± 115 µm (range, 326 to 729 µm). Therefore, this patient population had severe, chronic DME that was poorly responsive to standard therapies.

• **EFFECT OF RANIBIZUMAB ON FOVEAL THICKNESS:** Several patients had a large reduction in foveal thickness by seven days after the first intraocular injection of 0.5 mg

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