Oral Minocycline for the Treatment of Diabetic Macular Edema (DME): Results of a Phase I/II Clinical Study

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Purpose. Inflammation contributes significantly to the pathogenesis of diabetic macular edema (DME). In particular, retinal microglia demonstrate increased activation and aggregation in areas of DME. Study authors investigated the safety and potential efficacy of oral minocycline, a drug capable of inhibiting microglial activation, in the treatment of DME.

Methods. A single-center, prospective, open-label phase I/II clinical trial enrolled five participants with fovea-involving DME who received oral minocycline 100 mg twice daily for 6 months. Main outcome measurements included best-corrected visual acuity (BCVA), central retinal subfield thickness (CST), and central macular volume using spectral domain optical coherence tomography (SD-OCT) and late leakage on fluorescein angiography (FA).

Results. Findings indicated that the study drug was well tolerated and not associated with significant safety issues. In study eyes, mean BCVA improved continuously from baseline at 1, 2, 4, and 6 months by +1.0, +4.0, +4.0, and +5.8 letters, respectively, while mean retinal thickness (CST) on OCT decreased by −2.9%, −5.7%, −13.9, and −8.1% for the same time points. At month 6, mean area of late leakage on FA decreased by −34.4% in study eyes. Mean changes in contralateral fellow eyes also demonstrated similar trends. Improvements in outcome measures were not correlated with concurrent changes in systemic factors.

Conclusions. In this pilot proof-of-concept study of DME, minocycline as primary treatment was associated with improved visual function, central macular edema, and vascular leakage, comparing favorably with historical controls from previous studies. Microglial inhibition with oral minocycline may be a promising therapeutic strategy targeting the inflammatory etiology of DME. (ClinicalTrials.gov number, NCT01120899.) (*Invest Ophthalmol Vis Sci.* 2012;53:3865–3874) DOI:10.1167/iovs.11-9413

As the prevalence of diabetes mellitus increases worldwide, diabetic retinopathy (DR) has grown in global significance as a major cause of vision loss.[1,2] Diabetic macular edema (DME) is a common vision-threatening form of DR that develops in approximately 20% of diabetic patients within 15 years following the diagnosis of diabetes[3] and when untreated, culminates in progressive central vision loss with time.[4,5] While pathophysiological processes leading to DME are incompletely understood, it is clear that the pathology extends beyond a pure microvasculopathy to include other factors such as inflammatory mechanisms.[6] Features of chronic inflammation that are evident in DR include increased nitric oxide (NO) production, intracellular adhesion molecule-1 upregulation, leukostasis, and the release of pro-inflammatory cytokines, which are associated with deleterious effects such as vascular damage and neuronal cell loss.[6–8] As such, therapeutic approaches targeted at the inflammatory component of DME hold promise in reducing vision loss in diabetic patients.

Microglia, the primary resident immune cell of the retina, mediate and regulate multiple inflammatory changes and have been implicated in inflammatory changes underlying DR. Microglial alterations have been found in histopathological specimens of DR,[9] as well as in different mouse models of DR.[10–13] In diabetic retinas, microglia proliferate, demonstrate activated morphologies, upregulate expression of inflammatory cytokines, and aggregate near DR lesions, including perivascular areas and areas of cystic retinal edema.[9] Evidence that activated retinal microglia may actually contribute to DR progression has been found in animal studies where interventions that decrease microglial activation were successful in alleviating pathological features of DR.[12–14]

For these reasons, retinal microglia represent a promising cellular target for therapies that aim to limit the deleterious inflammatory changes in DR. While therapeutic agents that specifically inhibit the signaling of individual proinflammatory molecules may have efficacy, an approach that targets the activation of the cellular mediator, the microglia cell, may reduce the production of multiple relevant proinflammatory molecules, resulting in broader and more durable therapeutic effects. Minocycline, a commonly used second-generation tetracycline, has been demonstrated in cell culture and animal models to have anti-inflammatory properties that are independent of its antibacterial property. It has high bioavailability, readily crosses the blood-CNS barrier, and has been used in a number of human and animal models of neuroinflammation where microglia are implicated.[15–21] In a mouse model of diabetes, minocycline has been demonstrated to decrease diabetes-induced inflammatory cytokine production, reduce the release of cytotoxins from activated microglia, and significantly reduce apoptosis in the retina.[13] However, a proof-of-concept clinical study targeting microglial activation as a therapeutic strategy has not previously been performed. This current study presents results of a phase I/II pilot proof-of-concept study investigating the safety and potential efficacy of oral minocycline as a treatment that targets activated microglia in DME.

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Materials and Methods

This was a prospective, nonrandomized, uncontrolled, single-center, phase I/II pilot study that investigated the safety and potential efficacy of minocycline as a treatment for patients with DME. The research was supported by the Intramural Research Program at the National Eye Institute. The study protocol adhered to the tenets of the Declaration of Helsinki. The study protocol and informed consent forms were approved by an NIH-based institutional review board and the study was registered at www.clinicaltrials.gov (NCT01120899).

Eligibility Criteria

Participants were enrolled according the following person-based inclusion criteria: (1) at least 18 years of age, (2) diagnosis of type 1 or type 2 diabetes, and (3) medically stable with normal renal and hepatic function. Patients that were medically unstable, allergic to minocycline or fluorescein, or unwilling to use birth control while being of childbearing potential were excluded from enrollment. In addition, participants were required to have at least one eligible study eye, as defined by the following criteria: (1) best-corrected Early Treatment of Diabetic Retinopathy Study (ETDRS) visual acuity score between 78 and 39 letters (i.e., between 20/32 and 20/200); (2) retinal thickening involving the center of the fovea (defined as central subfield thickness [CST] on baseline optimal coherence tomography [OCT] measurement >250 microns) secondary to DME; and (3) history of previous treatment with focal laser photocoagulation following standard-of-care/best practice guidelines unless DME had been determined by the treating investigator as either not responsive or not amenable to laser treatment. Eyes that met any of the following criteria were excluded from enrollment: (1) had severe disease that were judged by the treating investigator as being unlikely to benefit from further therapy (such as those with central ischemia or macular scarring); (2) had vision loss from other coexisting ocular disease; (3) were treated with panretinal scatter photocoagulation (PRP) within 4 months prior to study entry; (4) had undergone pars plana vitrectomy within 6 months prior to study entry; (5) had undergone ocular surgical interventions (including cataract extraction, scleral buckle, and other intraocular surgery) within 3 months prior to study entry; (6) had undergone YAG capsulotomy within 2 months prior to study entry; and (7) had undergone treatments for DME such as intravitreal or periocular steroids or intravitreal anti-VEGF agents within 3 months prior to study entry.

Both eyes of each enrolled participants were evaluated in the study; one eye was designated as the "study eye" while the other was designated the "fellow eye." If only one eye in a participant fulfilled the inclusion criteria, that eye was designated the study eye. If both eyes met eligibility criteria, the treatment-naïve eye was designated as the study eye. If both eyes were naïve to treatment, the eye with the higher visual acuity score was designated as the study eye.

Study Drug

Minocycline hydrochloride (Ranbaxy Pharmaceutical Inc, Princeton, NJ; National Drug Code 63304 696 50) was reformulated by the NIH Research Pharmacy as capsules for oral administration. Each capsule contained 100 mg of minocycline with the following inactive ingredients: magnesium stearate and starch (corn), gelatin, silicon dioxide, sodium lauryl sulfate, titanium dioxide, and black iron oxide. Participants were instructed to take 100 mg of minocycline orally two times a day, once in the morning and once in the evening, approximately 12 hours apart.

Study drug compliance was monitored during the study. Participants were asked to record study drug administration using a "pill diary" and to return any unused study medication. Compliance data was obtained from a review of the pill diary at each study visit and from study drug accounting of unused medication. Unused study drug was returned to the NIH Research Pharmacy.

Study Design and Procedures

Five participants with DME were enrolled into the study according to the study eligibility criteria and were treated with 100 mg of minocycline orally twice daily for up to 24 months. Study visits were scheduled at baseline, month 1, month 2, and every 2 months thereafter until month 24. Additional ad hoc visits were permitted as clinically warranted.

Participants were evaluated at the baseline study visit with a medical history, review of systems, medication assessment, thyroid palpation, and serum blood analysis including hemoglobin A1c (HgbA1c), complete blood count, electrolyte analysis, and liver and thyroid function tests. Serum blood analyses were repeated at month 2 and every 4 months thereafter. Review of systems, adverse event assessment, and urine pregnancy testing (for female participants of childbearing age) were performed at each study visit.

Participants were evaluated at each study visit with a complete ophthalmic examination that included bilateral assessment of best-corrected visual acuity (BCVA), intraocular pressure measurement, and stereoscopic funduscopy. Best-corrected distance visual acuity was assessed using a standard ETDRS protocol and scored using the ETDRS logMAR visual acuity chart.

Spectral domain optical coherence tomography (SD-OCT) imaging was obtained in both eyes of each participant at each study visit. SD-OCT images were captured with an OCT instrument (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA) using the 512 × 128 scan pattern with the center of the 6 × 6-mm scanning area positioned at the center of the macula. Quantitative longitudinal analysis of OCT scans was performed by first aligning the scans spatially using functions provided within the OCT instrument software (Carl Zeiss) and were then checked for accuracy. The accuracy of automated delineations of the inner and outer retinal boundaries was also manually verified. OCT retinal thickness measurements in the macula were analyzed using a circular ETDRS-type grid positioned on the center of the fovea. Mean thickness measurements for the central subfield (central circle of diameter 1 mm) and for the four "inner" quadrants (circumscribed by a circle 3 mm in diameter, concentric to the central region and divided into superior, inferior, nasal, and temporal quadrants) were calculated. The volume of the retina summed over all five subfields, termed central macular volume (CMV) was also computed in units of cubic millimeters.[3]

Imaging by color fundus photography and fluorescein angiography (FA) was obtained a standard digital imaging system (Ophthalmic Imaging Systems Inc., Sacramento, CA) in both eyes of each participant at baseline and at month 6, month 12, month 18, and month 24. The area of late fluorescein leakage (at approximately 10 minutes) was graded in each eye by three independent retinal specialists (CC, WW, and EC) using a region-of-interest tool in an image analysis software package (ImageJ; NIH, Bethesda, MD). Graders were masked to the time point and identity of the images during grading. The measurements from each grader were then averaged and changes in the area of leakage from baseline calculated.

Study Outcomes

The primary outcome of the study is the change in visual acuity score in ETDRS letters from baseline at the month 6 visit. Secondary outcome measures include change from baseline in retinal thickness and macular volume as evaluated by SD-OCT, and the change in the area of late leakage as evaluated by FA at month 6 and every 6 months thereafter. Significant changes in OCT central subfield retinal thickness (CST) were determined using the logOCT scale[22] (where a change of >1 logOCT step is considered clinically significant. Change in logOCT is calculated using the following formula: Change in logOCT = log[CST at follow-up visit/200] – log[CST at baseline visit/200]).

At the month 4 visit, participants were assessed for "disease worsening," defined as either a decrease in visual acuity score from baseline of ≥15 ETDRS letters or a ≥50% increase in CST. Participants

Table 1. Baseline Demographics and Medical Characteristics of Study Participants ($n = 5$)

Patient	Sex	Age (years)	Race	Duration of Type II Diabetes (years)	Insulin Use (Yes/No)	HgbA1C (%)	Blood Pressure (mm Hg)	Serum Creatinine (mg/dL)
1	F	71	Black	11	Yes	7.8	164/68	0.91
2	F	61	Black	21	Yes	7.8	120/60	0.99
3	M	50	White	17	Yes	10	100/73	0.8
4	M	64	White	16	No	7.1	146/67	1.1
5	M	78	White	21	Yes	7.5	122/68	0.97
Mean		65.3		17		8.0	130/67	0.95
SD		3.3		4		1.1	25/5	0.11

SD, standard deviation.

meeting criteria for disease worsening were permitted to undergo adjunctive therapy (focal laser therapy for any amenable leaking microaneurysms and/or anti-VEGF intravitreal injections as appropriate) in addition to the study drug. At all study visits at and subsequent to month 6, participants were evaluated for "disease improvement," defined as either a decrease in excess retinal thickening from baseline of >1 step on the logOCT scale (equivalent to >20% decrease in CST) or a ≥15 ETDRS letter increase in visual acuity score from baseline. Eyes in the study that failed to improve sufficiently to criteria for disease improvement were offered adjunctive therapy (focal laser therapy for any amenable leaking microaneurysms and/or anti-VEGF intravitreal injections as appropriate) in addition to the study drug. The frequency of focal laser treatment in any eye in the study was limited to once every 4 months in accordance with standard-of-care/best practice guidelines.

Results

Study Participants and Study Eyes

Five participants were enrolled between June 2010 and February 2011 and all completed scheduled study visits up to month 6. The participants' baseline demographic and medical characteristics are summarized in Table 1. Participants ranged in age from 50 to 78 years (65 ± 3 years, mean ± SD) and had been diagnosed with type 2 diabetes for greater than 10 years (11–21 years, range) at study baseline. Three participants were Caucasian males and two participants were African American females. Baseline HgbA1C values ranged from 7.1% to 10%. Four of five participants had a history of hypertension, one had a history of a myocardial infarction, and all five had a history of hypercholesterolemia.

Baseline characteristics of the eyes of study participants ($n = 10$) are summarized in Table 2. As described in the "Methods" section, one eye of each participant was designated as the study eye, while the other eye was designated as the fellow eye. Three out of five fellow eyes also met eye-specific criteria for inclusion and were described as "qualifying eyes." Visual acuity in study eyes ranged from 57 to 77 letters (approximately 20/32 to 20/63), with a mean of 65 ± 9 letters (Snellen equivalent 20/40). All (5/5) study eyes had previously been treated with focal laser; 2/5 eyes had received prior treatment with intravitreal bevacizumab; 2/5 eyes had been treated with panretinal photocoagulation (PRP); and 1/5 eyes received intravitreal triamcinolone (all >3 months prior to baseline visit). Mean CST at baseline in the study eye ranged from 275 to 527 μm (440 ± 100 μm, mean ± SD).

Beginning from the month 4 visit, all eyes in the study were evaluated for needing adjunctive treatment using criteria for disease worsening and disease improvement. At the month 4 visit, none of the study and fellow eyes met criteria for disease worsening and was able to continue on study drug without additional adjunctive treatment up to the month 6 visit.

Ocular and Systemic Safety of Study Drug

The study drug was generally well tolerated. A total of 14 adverse events (AE) were recorded from baseline to the month 6 visit. All but one AE were mild in severity: one severe AE of laryngeal cancer was recorded and was considered unrelated to the study drug. Mild drug-related AEs included diarrhea, dizziness, and vaginitis. No ocular AEs were reported. The details of the AEs are summarized in Table 3.

Table 2. Baseline Ocular Characteristics of Study Participants ($n = 5$)

Patient	Study Eye Assignment	Visual Acuity (ETDRS letters, Snellen equivalent)		Prior Treatments for DR		OCT Thickness, Central Subfield (μm)	
		Study Eye	Fellow Eye	Study Eye	Fellow Eye	Study Eye	Fellow Eye
1	OS	60, 20/63	54, 20/80	Focal laser	Focal laser, bevacizumab	502	431
2	OD	72, 20/32	77, 20/32	Focal laser, PRP	Focal laser, PRP	527	495
3	OD	77, 20/32	85, 20/20	Focal laser	None	275	244
4	OS	57, 20/63	75, 20/32	Focal laser, bevacizumab, PRP	Focal laser, bevacizumab	425	280
5	OS	61, 20/63	80, 20/25	Focal laser, bevacizumab, intravitreal triamcinolone	Focal laser	470	312
Mean		65, 20/50	74, 20/32			440	352
SD		8.6	11.9			99.7	106.2

DR, diabetic retinopathy; ETDRS, Early Treatment of Diabetic Retinopathy Study; OCT, optical coherence tomography; OD, right eye; OS, left eye; PRP, pan-retinal photocoagulation; SD, standard deviation.

Table 3. Summary of AEs by Category and Severity for All Participants ($n = 5$)

	Severity					
	Mild/Grade 1		Severe/Grade 3		Total	
Adverse Event Category	*n*	%	*n*	%	*n*	%
General	2	14.3	0	0.0	2	13.3
Blood and lymphatic	2	14.3	0	0.0	2	13.3
Endocrine	1	7.1	0	0.0	1	6.7
Genitourinary	2	14.3	0	0.0	2	13.3
Gastrointestinal	4	28.6	0	0.0	4	26.7
Metabolism and nutrition	0	0.0	0	0.0	0	0.0
Musculoskeletal	1	7.1	0	0.0	1	6.7
Neoplasms: benign, malignant, and unspecified	0	0.0	1	100.0	1	6.7
Nervous system	2	14.3	0	0.0	2	13.3
Ocular	0	0.0	0	0.0	0	0.0
Pulmonary	0	0.0	0	0.0	0	0.0
Skin	0	0.0	0	0.0	0	0.0
Total	14	100.0%	1	100.0	15	100.0

Change in BCVA

The primary outcome measure of the study was specified as the change in BCVA in the study eye from baseline evaluated at month 6. Figure 1A shows the change in BCVA from baseline in all five study and fellow eyes at each study visit (month 1, month 2, month 4, and month 6). At the month 6 visit, 5/5 study eyes and 4/5 fellow eyes demonstrated an increase in BCVA from baseline; the only fellow eye that did not demonstrate an increase in BCVA had a baseline BCVA of 85 letters (20/20), which remained stable up to month 6. The net change in BCVA for each eye in the study from baseline to month 6 is summarized in Table 4.

The mean changes in BCVA from baseline for all study eyes ($n = 5$) and fellow eyes ($n = 5$) are shown in Figure 1B. The mean change in BCVA for all eyes in the study ($n = 10$) and for all eyes meeting eligibility criteria (i.e., qualifying eyes; $n = 8$) are shown separately in Figure 1C. From baseline to month 6, mean BCVA change demonstrated a progressive increase as a function of follow-up time. At month 6, the mean change in BCVA from baseline was 5.8 ± 5.4 letters for study eyes ($P = 0.074$, paired *t*-test, $n = 5$), and 4.4 ± 3.5 letters for fellow eyes ($P = 0.049$, paired *t*-test, $n = 5$). When combining study and fellow eyes, the mean change in BCVA from baseline was 5.1 ± 4.4 for all eyes ($P = 0.005$, paired *t*-test, $n = 10$), and 5.6 ± 4.5 letters for qualifying

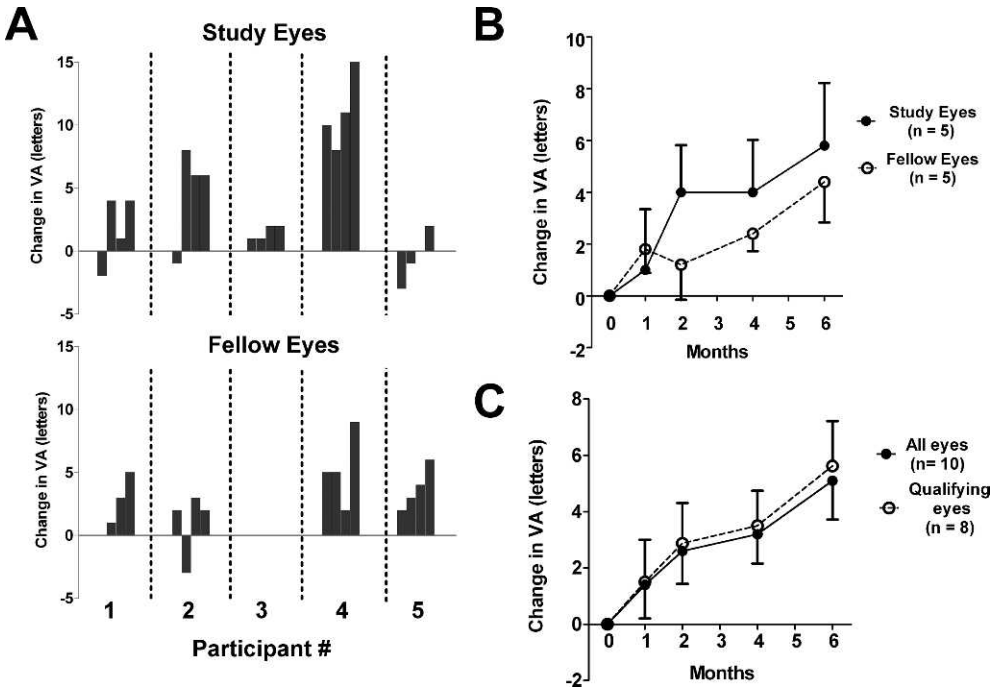

Figure 1. Change in BCVA from baseline in participants ($n = 5$) with DME. (**A**) Summary of visual acuity changes from baseline for study eyes ($n = 5$, *top panel*) and fellow eyes ($n = 5$, *bottom panel*) for all study visits. Each histogram column shows the change in visual acuity score (in ETDRS letters) for all study visits (month 1, month 2, month 4, and month 6) arranged in consecutive order. (**B**) Mean change in visual acuity score from baseline over time for study eyes ($n = 5$, *solid symbols*) and fellow eyes ($n = 5$, *open symbols*). (**C**) Mean change in visual acuity score from baseline over time for all eyes in the study (study eyes and fellow eyes combined, $n = 10$, *solid symbols*) and for qualifying eyes that met inclusion criteria at baseline ($n = 8$, *open symbols*). Error bars represent standard error.

Table 4. Correlation of Changes in Visual Acuity and Retinal Thickness Measurements to Other Patient Characteristics

Participant	Drug Adherence	Duration of Type II Diabetes (years) – All Type 2	HgbA1c (mg/dL)			Creatinine (mg/dL)			Blood Pressure (mm Hg)				Visual Acuity (Letters)				OCT CST (μm)		
			BL	Month 6	Change	BL	Month 6	Change	BL	Month 6	Change in Systolic	Change in Diastolic		BL	Month 6	Change	BL	Month 6	Change (%)
1	82%	11	7.8	7.2	−0.6	0.91	0.66	−0.25	164/68	140/68	−24	0	Study Eye	60	64	4	502	361	−28.1
													Fellow Eye	54	59	5	431	415	−3.7
2	97%	21	7.8	9.6	1.8	0.99	0.61	−0.38	120/60	143/71	23	11	Study Eye	72	78	6	527	435	−17.5
													Fellow Eye	77	79	2	495	297	−40.0
3	92%	17	10	9.1	−0.9	0.8	0.75	−0.05	100/73	135/79	35	6	Study Eye	77	79	2	275	278	1.1
													Fellow Eye	85	85	0	244	257	5.3
4	99%	16	7.1	6.4	−0.7	1.1	1.06	−0.04	146/67	137/58	−9	−9	Study Eye	57	72	15	425	460	8.2
													Fellow Eye	75	84	9	280	316	12.9
5	78%	21	7.5	6.4	−1.1	0.97	1.09	0.12	122/68	108/64	−14	−4	Study Eye	61	63	2	470	451	−4.0
													Fellow Eye	80	86	6	312	293	−6.1
Mean	90%	17.2			−0.3			−0.12			2.2	0.8				5.1			−7.2
SD	9.1%	4.1			1.2			0.2			25.4	7.9				4.4			16.7

BL, baseline visit; SD, standard deviation.

eyes ($P = 0.0095$, paired *t*-test, $n = 8$). While almost all eyes in the study demonstrated functional improvement, changes in BCVA were generally modest up to month 6, with only 1/5 study eyes demonstrating a >15-letter (3-line) increase.

Changes in OCT Macular Thickness and Volume Measurements

OCT imaging was obtained at all study visits in both eyes of all participants. Figures 2 and 3 depict the central horizontal B-scan traversing the fovea for all study visits for study and fellow eyes, respectively. The top panels show the horizontal B-scans traversing the center of the central ETDRS circle at the fovea for consecutive study visits. The bottom panels show the topographic maps of the en-face view of the central macula, as constructed from 512 × 128 volume scans, at baseline and at month 6. Quantitative analysis of retinal thickness was evaluated in the central macula using the ETDRS circles of 1 mm and 3 mm diameter centered on the fovea. Figure 4A shows the changes in central subfield retinal thickness (CST) from baseline in all individual five study and fellow eyes at each study visit (month 1, month 2, month 4, and month 6). Participants 1, 2, and 5 showed decreases in CST in both the study and fellow eye at all visits up to month 6. Participant 3, who had the lowest CSTs in both the study and fellow eyes at baseline, demonstrated minimal change in CST from baseline to month 6. In the study eye of participant 4, CST decreased at month 4 but increased at month 6.

The mean percentage changes in CST from baseline for all study eyes ($n = 5$) and fellow eyes ($n = 5$) are shown in Figure 4B. The mean percentage change in CST for all eyes in the study ($n = 10$) and for all eyes meeting eligibility criteria (i.e., qualifying eyes; $n = 8$) are shown separately in Figure 4C. At month 6, the mean percentage change in CST from baseline was –8.1 ± 14.6% for study eyes ($P = 0.28$, paired *t*-test, $n = 5$); –6.5 ± 19.9% for fellow eyes ($P = 0.50$, paired *t*-test, I = 5); –7.3 ± 16.5% for all eyes ($P = 0.20$, paired *t*-test, $n = 10$); and –9.1 ± 17.9% for qualifying eyes ($P = 0.19$, paired *t*-test, $n = 8$). All comparisons for all the subset of eyes had a *P* value >0.05 (paired *t*-test). As for visual acuity, mean percentage change in CST demonstrated a decreasing trend as a function of study time (Figs. 4B, 4C).

CMV, defined as the overall retinal volume within the 3-mm diameter circle of the ETDRS, was also computed and analyzed. The mean percentage changes in CMV from baseline are shown (Figs. 5A, 5B) and demonstrated similar decreasing trends as a function of follow-up time as those seen in changes in CST.

Changes in Vascular Permeability in terms of Leakage on FA

FA was obtained in study participants at baseline and at month 6. Early (1–3 minutes) and late phases (≈10 minutes) of the angiogram were examined and compared in both study and fellow eyes (Figs. 6A, 6B). The areas of late leakage were independently measured by three masked graders and changes from baseline at month 6 computed for each participant in terms of the absolute change in area (in μm^2) (Fig. 6C) and percentage change in area (Fig. 6D). These comparisons show that 5/5 study eyes and 4/5 fellow eyes demonstrated decreases in the area of late leakage.

Correlation of Visual Acuity and OCT Changes to Other Patient Characteristics

Changes in systemic measures and their relationship to changes in ocular findings were also examined in study

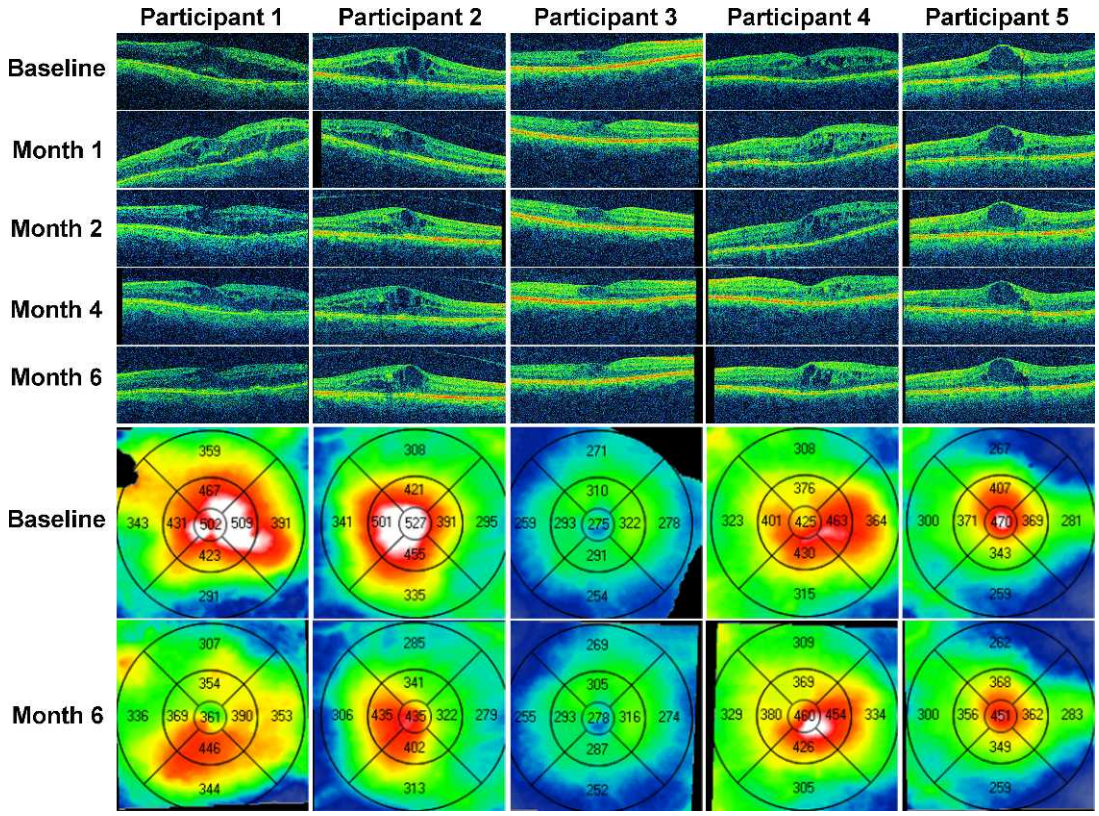

Figure 2. Change in macular thickness and edema in study eyes ($n = 5$) as evaluated by OCT analysis. *Upper panel*: Horizontal B-scans traversing the center of the central ETDRS circle at the fovea for all study visits. Consecutive images illustrate progressive anatomical changes at the same horizontal locus of the macula as a function of time. *Lower panel*: Topographic maps of the en-face view of the central macula, as constructed from 512 × 128 volume scans, at baseline and at month 6. The maps are overlaid with an ETDRS grid and the numbers in each sector of the grid represent mean retinal thickness in that sector in μm. All scans and topographical maps have been checked for alignment and correct delineation to allow accurate comparisons in corresponding retinal loci. Study eyes in participants 1, 2, and 5 demonstrated the highest CST at baseline, which decreased at month 6. Study eye in participant 4 decreased progressively in central macular edema up to month 4, which increased again at month 6. Study eye in participant 3 which had the smallest amount of central edema remained relatively unchanged across all study visits.

participants (Table 4). Serum creatinine demonstrated minimal changes over the study period for all five participants (–0.12 ± 0.2 mg/dL, mean ± SD). Measures of HgbA1c demonstrated more varied changes over 6 months with two participants having values that differed by more than 1% between baseline and 6 months. Interestingly, reductions in HgbA1c did not correlate to improvements in macular thickness or visual acuity. Participant 2 demonstrated an increase in HgbA1c of +1.8%, but also showed marked improvements in visual acuity and macular edema in both the study and fellow eye. On the other hand, participant 5 showed a decrease in HgbA1c of −1.1%, but only modest changes in visual acuity and macular thickness. Similarly, no consistent relationship was found between changes in blood pressure and ocular outcome measures.

DISCUSSION

In this prospective, five-participant, phase I/II pilot study, minocycline administered orally at a dose of 100 mg taken twice daily achieved a relatively high drug adherence rate (90 ± 9%, mean ± SD; range = 78%–99%) among participants during the study. The study drug was well tolerated, with minimal drug-related AEs or ocular complications. At month four, 5/5 participants did not meet criteria for disease worsening, and therefore did not require ancillary treatment for DME. As a result, all five participants were treated with minocycline only from baseline to month 6.

From baseline to month 6, mean visual acuity and mean central macular thickness and volume improved progressively with time in study and fellow eyes. While the improvements in visual acuity changes were modest overall (5.8 ± 5.4 in study eyes and 4.4 ± 3.5 in fellow eyes), all but one eye (9/10) demonstrated improvement in acuity; 1/10 eye remained stable at 85 letters (20/20); and 0/10 eyes demonstrated a decrease in visual acuity. One participant met the primary outcome measure of improvement in visual acuity by 15 letters (participant 4). The progressive improvements in visual acuity were generally concurrent with progressive decreases in macular edema as measured by CST and CMV, with some exceptions. The study eye in participant 4 demonstrated a consistent and durable improvement in visual acuity from baseline to month 6, even though improvements in macular edema measurements were more variable over follow-up. Of the eyes in the study, only one study eye (in participant 1) demonstrated a decrease in CST that exceeded 1 logOCT step. On FA, 9 out of 10 eyes demonstrated a decrease in the area of late leakage at month 6.

Taken together, these findings indicate a potential effect of the study drug in reducing abnormal vascular permeability, and thereby improving macular edema. Increased vascular permeability can be related to the presence of activated retinal microglia, which produce a host of inflammatory mediators, including TNF-α, interleukin-1β, intercellular adhesion mole-

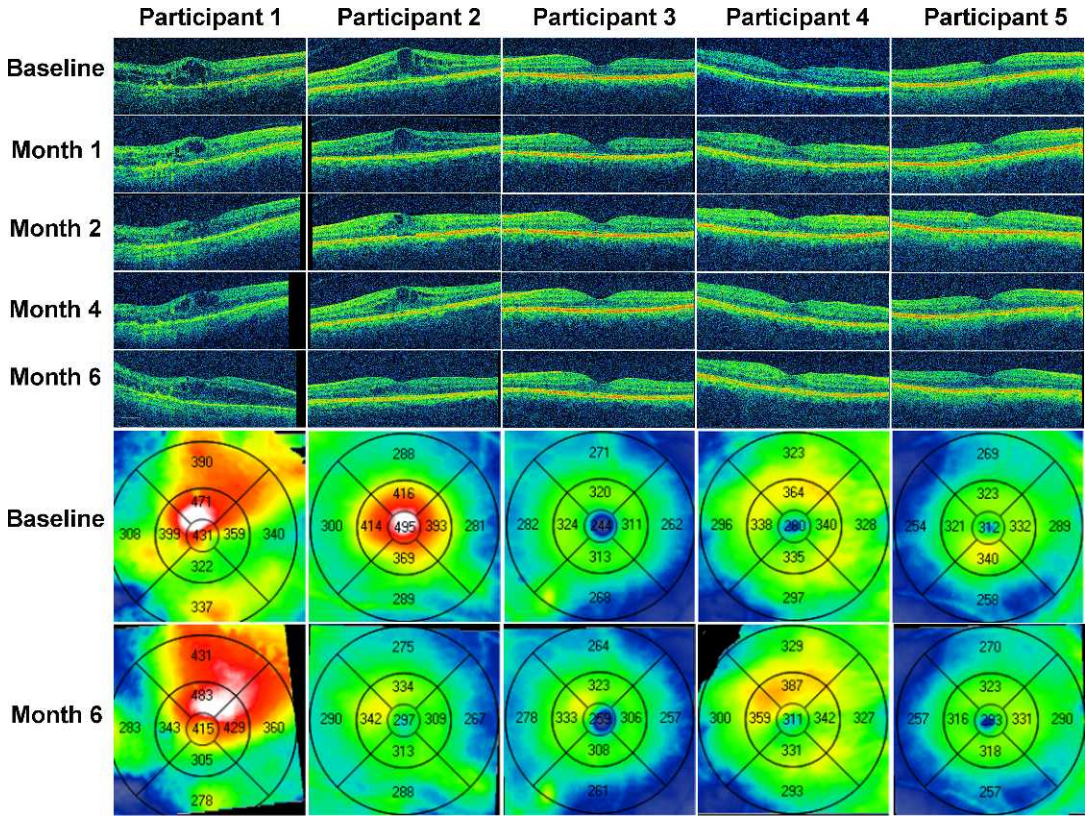

Figure 3. Change in macular thickness and edema in fellow eyes ($n = 5$) as evaluated by OCT analysis. *Upper panel*: Horizontal B-scans traversing the center of the central ETDRS circle at the fovea for all study visits. Consecutive images illustrate progressive anatomical changes at the same horizontal locus of the macula as a function of time. *Lower panel*: Topographic maps of the en-face view of the central macula, as constructed from 512 × 128 volume scans, at baseline and at month 6. The maps are overlaid with an ETDRS grid and the numbers in each sector of the grid represent mean retinal thickness in that sector in μm. All scans and topographical maps have been checked for alignment and correct delineation to allow accurate comparisons in corresponding retinal loci. Fellow eyes in participants 3 and 5 did not meet enrollment criteria and are considered "non-qualifying eyes." These participants showed five relatively mixed trends with time.

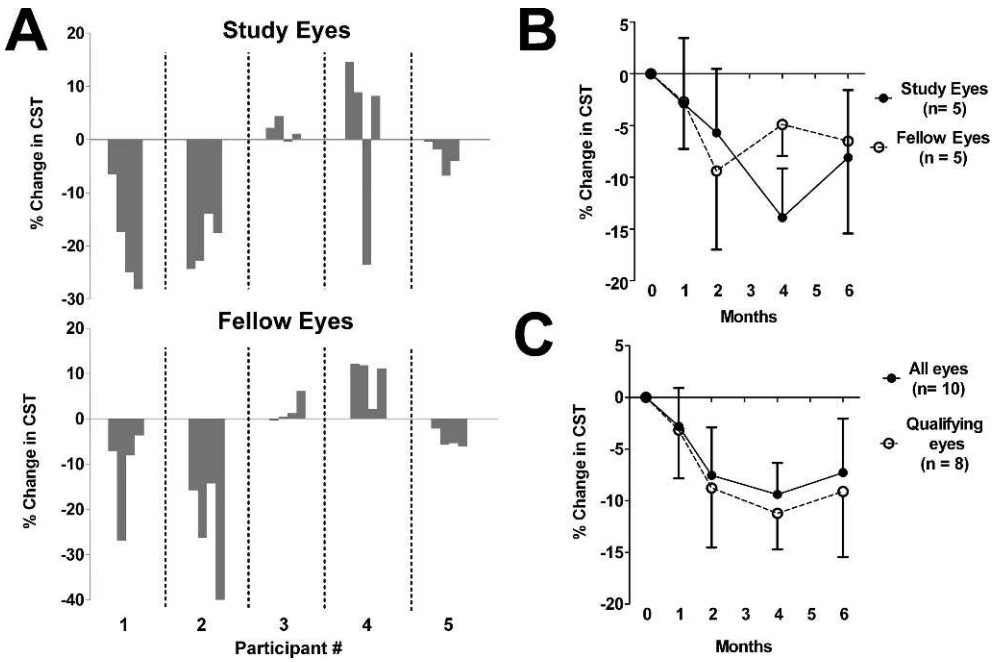

Figure 4. Percentage change in CST from baseline in participants ($n = 5$) with DME as measured using OCT. (**A**) Summary of visual acuity changes from baseline for study eyes ($n = 5$, *top panel*) and fellow eyes ($n = 5$, *bottom panel*) for all study visits. Each histogram column shows the percentage change in CST for all study visits (month 1, month 2, month 4, and month 6) arranged in consecutive order. (**B**) Mean percentage change in CST from baseline over time for study eyes ($n = 5$, *solid symbols*) and fellow eyes ($n = 5$, *open symbols*). (**C**) Mean percentage change in CST from baseline over time for all eyes in the study (study eyes and fellow eyes combined, $n = 10$, *solid symbols*) and for "qualifying eyes" that met inclusion criteria at baseline ($n = 8$, *open symbols*). Error bars represent standard error.

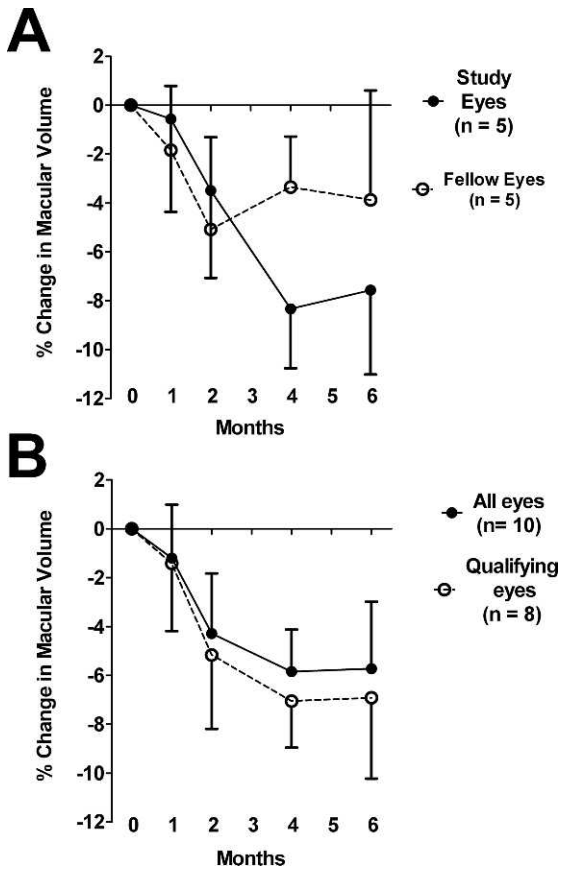

Figure 5. Percentage change in CMV from baseline in participants ($n = 5$) with DME as measured using OCT. Summary of change in CMV in eyes relative to baseline. (**A**) Mean percentage change in CMV from baseline over time for study eyes ($n = 5$, *solid symbols*) and fellow eyes ($n = 5$, *open symbols*). (**B**) Mean percentage change in CMV from baseline over time for all eyes in the study (study eyes and fellow eyes combined, $n = 10$, *solid symbols*) and for "qualifying eyes" that met inclusion criteria at baseline ($n = 8$, *open symbols*). Error bars represent standard error.

cule 1, cyclooxygenase, inducible nitric oxide synthase, and VEGF, which can induce retinal leukostasis and blood-retinal barrier breakdown.[7] Although the initial stimulus for microglial activation is unclear, sustained microglial activation in the retina at sites of DR lesions can perpetuate chronic neuroinflammation and exacerbate DR-related pathological changes. In vitro, minocycline has been shown to inhibit the activation of microglia and effectively decrease the expression of inflammatory cytokines.[23] In vivo, systemic minocycline in a rodent model of diabetes similarly repressed diabetes-induced upregulation of inflammatory mediators.[13] As a result, the anatomical effects in terms of reduced edema and vascular leakage observed in the present study are likely a result of reduced microglial activation in the retina.

In addition to reducing vascular permeability and improving macular edema, microglia inhibition with minocycline may also act in other ways to improve visual acuity in DME. Activated microglia in the CNS can induce neuronal death[24] and synapse degeneration[25]; and in the diabetic retina, these effects may contribute to neurodegeneration, which may not be evident macroscopically on clinical examination.[10,26] In animal models of retinal diseases including glaucoma,[27] retinal hemorrhage,[28] and photoreceptor degeneration,[29,30] treatment with minocycline has resulted in a decrease in neuronal degeneration. Minocycline was also effective in reducing diabetes-induced upregulation of caspase-3, a mediator of apoptotic cell death in a rodent model of diabetic retinopathy.[13] These studies highlight the involvement of microglial-mediated chronic neuroinflammation in neuronal and synaptic dysfunctions in DR, and suggest an additional mechanism by which minocycline may contribute toward the improvements in visual acuity observed in the current study.

Although the current pilot phase II study did not contain a control arm, study authors compared clinical outcomes with available data from control groups from other clinical studies of diabetic macular edema. Comparisons were made taking into account the use of rescue laser treatment in these control groups, and the time following enrollment that measurements were made. The safety and efficacy of ranibizumab in diabetic macular edema with center involvement (RESOLVE) study, a sham-controlled, double-masked study of eyes with DME,[31] contained a sham-treatment control arm containing 49 eyes in which rescue laser was made available 3 months following enrollment. At the planned interim analysis of a subset of eyes at 6 months, mean OCT CST increased by 15% in the sham-treated group. Change in BCVA was not reported. At the 12-month primary outcome time point, the mean change in OCT CST was –48.4 ± 153.4 μm and the mean change in BCVA was –1.4 ± 14.2 letters. The phase III study of ranibizumab injection in subjects with clinically significant macular edema with center involvement secondary to diabetes mellitus (RISE) and the phase III study of ranibizumab injection in subjects with clinically significant macular edema with center involvement secondary to diabetes mellitus (RIDE) similarly included a sham-treatment control arm in which rescue laser was also available 3 months following enrollment. While the results of the RIDE and RISE studies have not been published, data was available from a presented abstract (Brown DM, et al. *IOVS* 2011;52: ARVO E-Abstract 6647), which reported that at 24 months, the control arm decreased in mean central foveal thickness by 133 μm, with 18% of participants improving by at least 3 lines of vision. Data from the ETDRS[32] also demonstrated that the subset of study eyes that were comparable in disease severity (i.e., with mild to moderate nonproliferative diabetic retinopathy and macular edema at the center of the macula), in which focal/grid laser was deferred, experienced a vision decrease from baseline of –0.9 ± 7.3 letters.

While the current study included the possibility of providing rescue laser, no participant reached the laser-rescue criteria in the first 6 months of the study. As such, all participants were receiving minocycline treatment only. Although study authors observed modest improvements in visual acuity and OCT thickness at 6 months, these changes compared favorably with those from the control cohort in the RESOLVE study at the 6- and 12-month time points. Comparisons with the control groups in the RIDE and RISE studies are complicated by the longer duration (24 months) of follow-up, across which rescue laser was additionally made available. As a result, the data here is interpreted as potentially revealing a positive effect secondary to study drug as suggested by: (1) the favorable comparison with the control group at 6 months in the RESOLVE study; (2) the use of study drug without additional ancillary treatment; (3) the time-dependent improvement of functional and anatomical outcome measures over the first 6 months; (4) the simultaneous improvements in mean BCVA, macular thickness, and fluorescein leakage; and (5) the absence of systemic trends (i.e., HgbA1C, blood pressure) that would suggest alternative etiologies for improvements in outcome measures.

While effective, current therapies for DME—such as intravitreal anti-VEGF agents and steroids and focal laser—are limited by a high burden of treatment, ocular adverse effects, and unclear mechanisms of action.[33] The therapeutic strategy

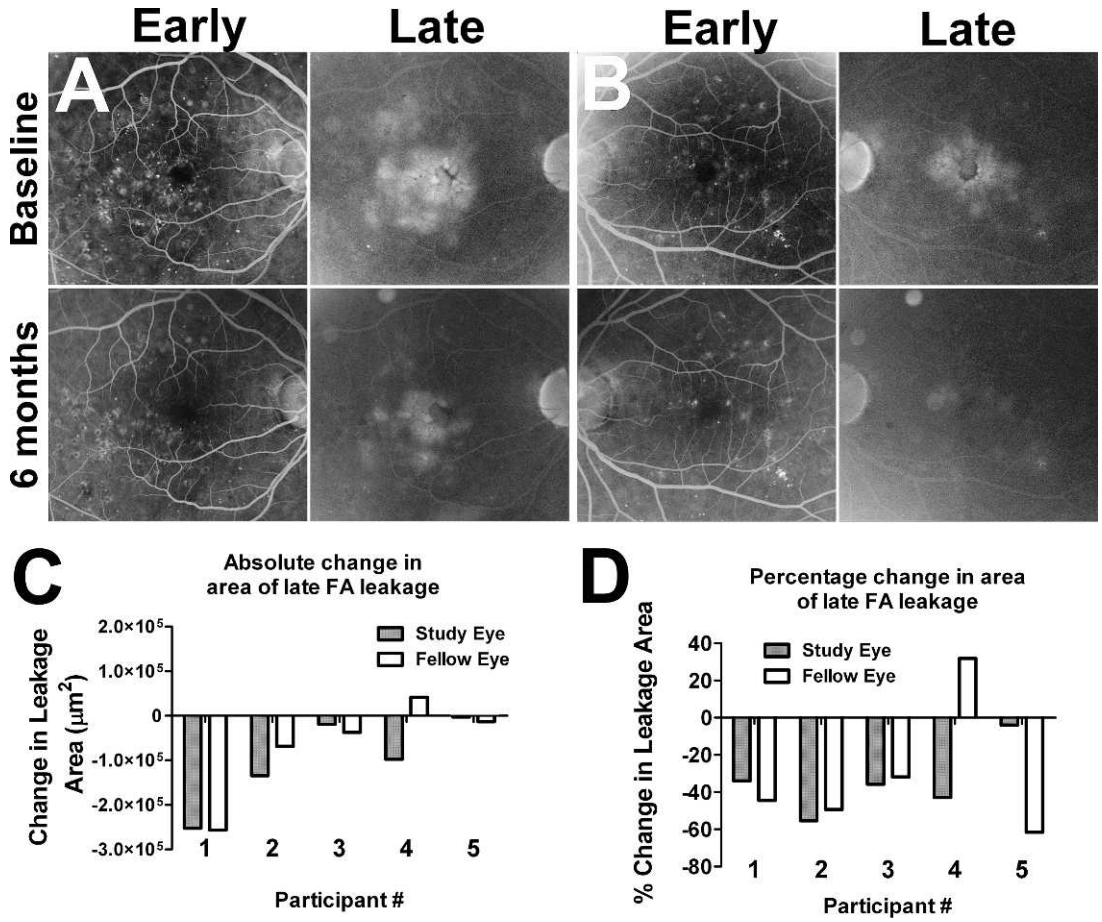

Figure 6. Changes in vascular permeability from baseline in participants ($n = 5$) with DME as measured using FA. Example of angiographic changes in the early (1–3 minutes) and late (≈10 minutes) phases of the angiogram as seen in participant 2 as examined at baseline (*top*) and month 6 (*bottom*). (**A**) Study eyes. (**B**) Fellow eyes. Comparisons of matched frames in this participant demonstrate decreases in the number of leaking microaneurysms seen in the early frames and in the area of late leakage in the late frames at month 6 relative to baseline. Quantifications of the changes in the area of late leakage from baseline to month 6 for all participants demonstrate net decreases in 5/5 study eyes and 4/5 fellow eyes. (**C**) Absolute changes. (**D**) Percentage changes.

of microglial inhibition may be a useful adjunct, as it broadly targets a central cellular mediator driving chronic neuroinflammation in DR Oral minocycline, with the advantages of high bioavailability, long history of use and known safety profile, and abundant preclinical data supporting its biological effects and its potential efficacy, is promising as a microglial-targeted therapy for DR and warrants further investigation.

In conclusion, the findings in this pilot proof-of-concept study indicate a potential effect of oral minocycline for the treatment of DME. Administered over 6 months, oral minocycline appeared to have potential efficacy in increasing visual acuity and reducing macular edema in a progressive time-dependent manner. These changes were associated with a decrease in vascular leakage as determined by fluorescein angiography and were not associated with concurrent changes in systemic factors such as glycemic index, blood pressure, or serum creatinine. The progressive improvement of outcomes measures with increasing duration of treatment was suggestive of a treatment effect that was secondary to the study drug. These findings encourage further investigation of the strategy of microglial inhibition with oral minocycline in the treatment of DME. These further studies may comprise of larger phase II trials in which minocycline or placebo may be assigned in combination with approved anti-VEGF therapies for DME, similar to pilot trials presently ongoing for the treatment of vein occlusions (NCT01468831 and NCT01468844). While current anti-VEGF therapies may strongly ameliorate VEGF-dependent pathological changes in the retina in diabetic retinopathy, they may not address the underlying etiology of VEGF dysregulation. Pharmacological strategies involving microglial inhibition hold promise in decreasing causative inflammatory changes and may constitute an ancillary treatment for reducing the chronicity of disease and the burden of treatment.

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