Effect of Doxycycline vs Placebo on Retinal Function and Diabetic Retinopathy Progression in Mild to Moderate Nonproliferative Diabetic Retinopathy: A Randomized Proof-of-Concept Clinical Trial

Ingrid U. Scott, MD, MPH; Gregory R. Jackson, PhD; David A. Quillen, MD; Ronald Klein, MD; Jason Liao, PhD; Thomas W. Gardner, MD, MS

IMPORTANCE Microglia have been associated with inflammatory changes underlying diabetic retinopathy.

OBJECTIVE To investigate whether low-dose oral doxycycline monohydrate, a drug capable of inhibiting microglial activation, can improve or slow the deterioration of retinal function and whether it can induce regression or slow progression of diabetic retinopathy in patients with mild to moderate nonproliferative diabetic retinopathy (NPDR).

DESIGN, SETTING, AND PARTICIPANTS Randomized, double-masked, 24-month proof-of-concept clinical trial. We randomized 33 patients (from the Penn State Hershey Eye Center) with at least 1 eye with mild to moderate NPDR (Early Treatment Diabetic Retinopathy Study level 20-43) to doxycycline monohydrate, 50 mg/d, or daily placebo for 24 months.

MAIN OUTCOMES AND MEASURES Mean change at 24 months compared with baseline in the foveal sensitivity of matrix frequency-doubling perimetry in each treatment group. We also compared the 2 groups with respect to change from baseline to 24 months in functional variables (Humphrey photopic visual field testing using the Swedish interactive thresholding algorithm 24-2 strategy, contrast sensitivity, dark adaptation, visual acuity, and quality of life) and anatomical variables (diabetic retinopathy severity level, area of retinal thickening, central subfield thickness on optical coherence tomography, and macular volume on optical coherence tomography).

RESULTS From baseline to month 24, no significant difference was detected between groups with respect to all visual function and anatomical outcomes assessed.

CONCLUSIONS AND RELEVANCE Although a link between low-dose oral anti-inflammatory agents and subclinical improvement in inner retinal function has been suggested in patients with severe NPDR or non–high-risk proliferative diabetic retinopathy, the same association was not found in the present study of patients with mild to moderate NPDR. The different findings in the 2 patient populations may relate to a differential effect of doxycycline on different stages of diabetic retinal dysfunction, or the sample size of the present study may be too small to detect a treatment effect of doxycycline in patients with mild to moderate NPDR.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT00917553

Published online June 26, 2014.
Diabetic retinopathy is the leading cause of visual impairment among working-aged adults. For patients with mild to moderate nonproliferative diabetic retinopathy (NPDR), there is currently no Food and Drug Administration-approved therapy known to prevent retinopathy progression.

A chronic low-grade retinal inflammatory response begins shortly after the onset of diabetes mellitus, and minocycline hydrochloride reduces this inflammation in diabetic rats. Low-dose tetracycline hydrochloride reduces connective tissue breakdown, protein glycation, and excessive connective tissue collagen synthesis in diabetes mellitus. Microglia, primary resident immune cells of the retina, become activated by diabetes mellitus and induce inflammatory changes underlying diabetic retinopathy. Tetracycline inhibits microglial-mediated cell death and retinal cell apoptosis and prevents retinal capillary damage via caspase inhibition. In an open-label phases 1 and 2 clinical study including 5 participants with fovea-involving diabetic macular edema, oral minocycline hydrochloride, 100 mg twice daily for 6 months, was associated with improved visual acuity and reduced central macular thickness and vascular leakage, comparing favorably with historical controls. Low-dose oral doxycycline monohydrate reduces systemic inflammation with no antibacterial effects. In a 24-month proof-of-concept clinical trial of patients with severe NPDR or non–high-risk proliferative diabetic retinopathy, doxycycline monohydrate, 50 mg/d, was associated with significantly improved foveal sensitivity compared with placebo. The purpose of the present proof-of-concept clinical trial is to investigate whether oral doxycycline, a drug capable of inhibiting retinal microglial activation, can (1) improve or slow deterioration of retinal function and (2) induce regression or slow progression of diabetic retinopathy in patients with mild to moderate NPDR and abnormal retinal function.

Methods

The study protocol was approved by the institutional review boards of the Penn State College of Medicine and University of Wisconsin School of Medicine and Public Health. Written and verbal informed consent was obtained from all participants. Patients were recruited from the Penn State Hershey Eye Center and included adults with diabetes mellitus and at least 1 eye with mild to moderate NPDR (Early Treatment Diabetic Retinopathy Study [ETDRS] levels 20-49) based on fundus photographs graded at the University of Wisconsin–Madison Reading Center and the absence of macular edema (central subfield thickness on optical coherence tomography, ≤275 μm). The study eye needed to demonstrate reduced retinal function (defined as a foveal sensitivity of ≤30.91 dB on matrix frequency-doubling perimetry [FDP]). We demonstrated previously that FDP foveal sensitivity is the most sensitive to NPDR of all visual function variables assessed in the present study, and a foveal sensitivity of 30.91 dB represents the 95% CI of normal FDP performance. Thus, patients with a foveal sensitivity of 30.91 dB or less were selected for the present study because they had the opportunity to improve their FDP performance or to progress to more severe impairment. Study eligibility criteria are listed in the Box.

The randomization scheme was a permuted block design, with patients stratified by dichotomized time since onset of diabetes mellitus (<10 years vs ≥10 years) and dichotomized hemoglobin A1c level (<9% vs ≥9% [to convert to a proportion of total hemoglobin level, multiply by 0.01]). Patients were randomized to doxycycline monohydrate, 50 mg/d, or an identical placebo, before meals for 24 months. Study investigators, coordinators, photographers, and patients were masked to treatment assignment.

At baseline, patients underwent complete ocular examinations, including best-corrected ETDRS visual acuity, intraocular pressure measurement, slitlamp and dilated funduscopy examinations, contrast sensitivity testing, visual field testing including FDP and Humphrey photopic visual field testing using the Swedish interactive thresholding algorithm 24-2, dark adaptation testing using a dark adaptometer (AdaptDx; MacuLogix, Inc), vision-specific quality-of-life assessment questionnaires (National Eye Institute Visual Function Questionnaire and Low-Luminance Questionnaire), time-domain optical coherence tomography (Stratus OCT; Carl Zeiss Meditech), modified 7-standard field color stereoscopic fundus photography, and fluorescein angiography. Detailed methods regarding these assessments have been published elsewhere.

Follow-up examinations (including all baseline assessments except fluorescein angiography) were performed at 6, 12, 18, and 24 months. Fluorescein angiography was performed at 24 months. All adverse events were recorded. Fundus photographs were evaluated at the Reading Center, and fluorescein angiography and optical coherence tomography images were reviewed by study investigators (I.U.S., G.R.J., D.A.Q., and T.W.G.). Because FDP foveal sensitivity has been demonstrated to be the most sensitive to NPDR of all visual function variables included in the present study, the primary outcome of the present study is the mean change in the FDP foveal sensitivity from baseline to 24 months in the doxycycline group compared with the placebo group.

Statistical Analysis

The sample size was based, in part, on administrative feasibility issues such as available funding. We compared outcomes in the 2 groups (adjusting for the baseline value of each variable) using the Fisher exact test for categorical characteristics or the Welch 2-sample t test for continuous measurements. We made no adjustment for multiple testing in this exploratory phase 2 study with small sample sizes. The statistical significance level for the primary outcome was set at $P < .05$.

Results

Seventeen participants were randomized to the placebo group and 16 to the doxycycline group. Two participants in the placebo group after month 18 and 1 participant in the doxycycline group after month 6 could not be located to complete documentation; the remaining 30 participants completed 24 months of follow-up. Demographic and baseline characteristics are summarized in Table 1. A significant difference between groups was not detected with respect to demographic and baseline characteristics.
**Box. Study Eligibility Criteria**

**Inclusion Criteria**
- Age ≥18 years
- Diagnosis of type 1 or type 2 diabetes mellitus (defined as current regular use of oral antihyperglycemics and/or insulin for the treatment of diabetes mellitus)
- Hemoglobin A1c level of ≤11% at prequalification visit
- Able and willing to give informed consent
- Best-corrected ETDRS VA in study eye of ≥69 letters (20/40 Snellen lines)
- Mild to moderate NPDR (ETDRS levels, 20-43) and in whom the need for retinal photocoagulation or other treatment for diabetic retinopathy (eg, intravitreal anti-VEGF or intravitreal corticosteroid) is not anticipated by the investigator within the subsequent 2 years
- Able to perform reliable visual field and dark adaptation testing
- Central subfield thickness on OCT of ≥275 μm
- Media clarity and pupil dilation sufficient for high-quality fundus photographs
- Abnormal retinal function measured as abnormal FDP function (ie, a foveal sensitivity of ≥30.91 dB)

**Ocular Exclusion Criteria**
- Prior panretinal photocoagulation in the study eye
- Prior focal/grid laser photocoagulation in the macula in the study eye
- Intraocular pressure in the study eye of ≥22 mm Hg by Goldmann tonometry
- History of pars plana vitrectomy in the study eye
- Systemic or intravitreal anti-VEGF agent to the study eye or the fellow eye within the past 3 months
- Peribulbar corticosteroid injection to the study eye or the fellow eye within the past 6 months
- Intravitreal triamcinolone acetonide to the study eye within the past 4 months
- Expectation by the investigator that retinal photocoagulation or other treatment for diabetic retinopathy (eg, focal/grid laser to the study eye, intravitreal triamcinolone to the study eye, intravitreal anti-VEGF agent to the study eye or the fellow eye, ruboxistaurin or systemic anti-VEGF agent for diabetic macular edema) will be administered in the subsequent 24 months
- An ocular condition (other than diabetes mellitus) present in the study eye that, in the opinion of the investigator, might alter VA during the course of the study (eg, retinal vein occlusion, uveitis, or other ocular inflammatory disease, neovascular glaucoma, Irvine-Gass syndrome, etc)
- History of major ocular surgery (including cataract surgery, scleral buckle, any intracocular surgery, etc) in the study eye within the prior 6 months or anticipated within the subsequent 24 months after randomization
- Aphakia in the study eye
- History of YAG capsulotomy performed in the study eye within 2 months before randomization

**Systemic Exclusion Criteria**
- Unstable medical status (eg, glycemic control, blood pressure, cardiovascular disease, individuals who are unlikely or unable to complete the 24-month trial) in the opinion of the investigator
- Significant renal disease (defined as a serum creatinine level of >2.5 mg/dL)
- Systolic blood pressure of >180 mm Hg or diastolic blood pressure of >110 mm Hg
- History of headaches associated with tetracycline hydrochloride therapy
- History of pseudotumor cerebri
- Pregnancy; for women of child-bearing potential, a pregnancy test will be performed
- Lactating or intending to become pregnant during the study period (≥24 months)
- Sexually active women of child-bearing potential not actively practicing birth control by using a medically accepted device or therapy (ie, intrauterine device, hormonal contraceptive, or barrier devices) during the study period (≥24 months); because doxycycline may interfere with the effectiveness of hormonal contraceptives, sexually active women of child-bearing potential who use a hormonal contraceptive will be required to use a second form of contraception to safeguard against contraceptive failure while participating in the study
- Known allergy/intolerance to doxycycline or any ingredient in the study drug or placebo (eg, cellulose, hypromellose, iron oxide, methacrylic acid copolymer, polyethylene glycol, polysorbate 80, sugar spheres, talc, titanium dioxide, and triethyl citrate)
- Patients taking phenytoin sodium, barbiturates, carbamazepine, digoxin, or isotretinoin, with gastroparesis, with a history of gastrectomy or gastric bypass surgery, or who were otherwise deemed achlorhydric because of altered doxycycline pharmacokinetics and/or bioavailability
- Patients taking strontium, acitretin, or tretinoin owing to the potential for serious drug interactions with doxycycline
- Patients with abnormal ALT or AST levels at baseline will be referred to their primary care physician for medical clearance for participation in this study

**Study Eye**
- If both eyes met study eligibility criteria, the eye with the more advanced ETDRS diabetic retinopathy level was selected to be the study eye
- If both eyes were judged to have the same diabetic retinopathy level, the eye with better VA was designated the study eye
- If both eyes had the same VA, the right eye was designated the study eye

**Table 2.** A significant difference between groups was not detected with respect to anatomical outcomes. At 24 months compared with baseline, the severity of diabetic retinopathy improved by at least 2 ETDRS diabetic retinopathy severity levels in none of the patients in the doxycycline group compared with 1 patient (6%) in the placebo group (P < .99). Analyses were...
also performed adjusting for baseline retinopathy level, and again no significant difference between groups was detected with respect to functional and anatomical outcomes.

Six serious adverse events were reported (ie, confusional state, angina, worsening arthritis, Charcot arthropathy, diabetic ketoacidosis, and umbilical hernia repair). No adverse events were considered related to the study drug.

### Discussion

In this 24-month proof-of-concept clinical trial among patients with mild to moderate NPDR, we detected no significant effect of doxycycline monohydrate, 50 mg/d, on retinal function or diabetic retinopathy. Study limitations include a...
small sample size, loss to follow-up of 3 participants, and a follow-up duration of only 24 months. In contrast to results of the present study, a significantly improved mean FDP foveal sensitivity was observed in the doxycycline group compared with the placebo group in a similar 24-month randomized trial in patients with severe NPDR or non-high-risk proliferative diabetic retinopathy. The different findings in the 2 studies may relate to a differential effect of doxycycline on different stages of diabetic retinal dysfunction. Perhaps doxycycline has a greater effect on diabetic retinal dysfunction in patients with more advanced diabetic retinopathy, and presumably more intraretinal inflammation, than in patients with earlier stages of diabetic retinopathy. Alternatively, the sample size of the present study may be too small to detect a doxycycline treatment effect. Finally, the different findings might result because analyses in eyes with more severe levels of retinopathy were performed without adjusting for baseline values of the variables. Further study to investigate the potential effect of low-dose oral anti-inflammatory agents on diabetic retinal dysfunction and diabetic retinopathy is warranted.

Conclusions

We found no association between low-dose oral anti-inflammatory agents and subclinical improvement in inner retinal function in the present study of patients with mild to moderate NPDR. Compared with a previous trial conducted in patients with severe NPDR or non–high-risk proliferative diabetic retinopathy, the different findings in the patient population of the present study may relate to a differential effect of doxycycline on different stages of diabetic retinal dysfunction. In addition, the sample size of the present study may be too small to detect a treatment effect of doxycycline in patients with mild to moderate NPDR. Further study to investigate the potential effect of low-dose oral anti-inflammatory agents on diabetic retinal dysfunction and diabetic retinopathy is warranted.


**OPHTHALMIC IMAGES**

**Optic Nerve Pit-Associated Choroidal Cleft**

Ruwan A. Silva, MD; Robert T. Chang, MD; Darius M. Moshfeghi, MD; Theodore Leng, MD, MS

*A, Posterior segment photograph of the left eye demonstrating a large inferotemporal optic nerve pit. Pigmentary mottling of the choroid is also noted. B, Spectral-domain optical coherence tomography through the optic pit reveals an associated choroidal cleft.*