Neuroprotection of Photoreceptors by Minocycline in Light-Induced Retinal Degeneration

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PURPOSE. Microglial cells have been found to play pivotal roles in various neuronal degenerative diseases such as Parkinson’s and Alzheimer’s diseases. Minocycline, a microglial inhibitor, has recently been shown to be neuroprotective in various models of cerebral ischemia and degenerative diseases of the brain. This study was conducted to evaluate the neuroprotective effect of minocycline and the role of microglia in light-induced retinal degeneration.

METHODS. BALB/cJ mice were exposed to intense green light for 3 hours and observed during 1, 3, or 7 days of dark recovery. The animals received intraperitoneal injections of minocycline or vehicle 1 day before exposure to light for 2, 4, or 8 days, depending on the periods of survival. Morphologic, morphometric, immunohistochemical, and electrophysiologic studies were performed to evaluate the efficacy of minocycline in the amelioration of light-induced retinal degeneration and the possible involvement of microglial cells.

RESULTS. Minocycline treatment provided marked amelioration in the loss of photoreceptors in light-induced retinal degeneration, as evidenced by morphologic, morphometric, and electrophysiologic criteria. Morphologically, the minocycline-treated group showed markedly better preservation of the outer retina after exposure to light. Morphometrically, at 7 days after exposure to light, in the minocycline-treated animals, 89.1% of the normal-appearing photoreceptor nuclei remained, but in the retinas of the vehicle-control group only 38.0% of these nuclei remained. This difference was statistically significant (P < 0.001). At 7 days after exposure to light electroretinography (ERG) showed that minocycline significantly preserved the amplitudes of dark-adapted a- and b-wave and light-adapted b-wave, which were all significantly reduced in the light-exposed, vehicle-treated control group (P < 0.01).

CONCLUSIONS. Minocycline is neuroprotective against light-induced loss of photoreceptors, possibly through the inhibition of retinal microglial activation. (Invest Ophthalmol Vis Sci. 2004;45:2753–2759) DOI:10.1167/iovs.03-1344

Microglial cells are resident immune surveillant cells in the central nervous system (CNS) and are believed to play an important role in modulating neuronal responses to insults.1–2 Activated microglial cells, which may be attracted and activated by chemokines, are known to produce cytotoxic factors that cause injury to neurons.3–6 In contrast to the many studies of the brain, there are few reports of studies that have examined the exact roles of these cells in retinal photoreceptor degeneration.7–11 In the retinal dystrophic Royal College of Surgeon (RCS) rats, microglia infiltrated the outer retina as the photoreceptor degenerated.7–9 These cells, when isolated from the retinas of RCS rats, were cytotoxic to photoreceptor cells in vitro.10 In light-induced photoreceptor degeneration, Ng and Streinlein11 demonstrated the presence of microglial cells in the subretinal space and their phagocytosis of degenerating photoreceptor cells. Our previous report showed significantly increased levels of chemokines in the light-induced retinal degeneration preceding to infiltration of activated retinal microglia into the outer retina (Zhang C, et al. IOVS 2003;43:ARVO E-Abstract 5122). Therefore, we hypothesized that in light-induced retinal degeneration, chemokines originated from the outer retina, activate and recruit from the inner retinal microglia, which in turn exaggerate the photoreceptor degenerative processes.

Consistent with the hypothesis that activated microglial cells are neurotoxic, several studies have shown that inhibition of microglial activation ameliorates neuronal degeneration.12–15 However, many of these inhibitors, such as macrophage inhibitory factor (MIF)12 and p38 mitogen-activated protein kinase (MAPK) inhibitors,13 have to be locally administered or applied in culture study because of their poor penetration of the blood–brain barrier. This limitation hinders the possible application of these inhibitors for clinical use. In contrast, minocycline, which is also an effective inhibitor of microglial activation, is capable of rapid penetration of the CNS. Several studies have shown its potent neuroprotective effects in animal models of global and focal cerebral ischemia and Parkinson’s disease.10–13 To examine the role of microglial activation and to explore the possible use of minocycline for neuroprotection on photoreceptors, we examined its effect on light-induced retinal degeneration using morphologic, morphometric, and electrophysiologic criteria.

MATERIALS AND METHODS

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the animal protocols were reviewed and approved by the Animal Care and Use Committees of The Johns Hopkins University and the University of Missouri–Columbia, respectively. Mice were exposed to light as described previously.20 Briefly, albino BALB/cJ male mice (Jackson
Laboratories, Bar Harbor, ME), aged 4 to 5 weeks, were housed under a 12-hour light–dark cycle with a light intensity of 15 lux for 7 days and dark-adapted for 2 days before exposure to light. Groups of animals were exposed to green-filtered fluorescent light (Plexiglas 2092 filter; Polycast Technology Corp., Stamford, CT) at a level of 3.5 klx for 3 hours. The filter transmitted light between 490 and 580 nm with a peak around 535 nm. Circular white fluorescent light tubes were installed outside the green-filtered plastic chamber to provide equal exposure in all directions inside the light chamber. Animals were euthanatized by cervical dislocation after deep anesthesia (intraperitoneal chloral hydrate; Sigma-Aldrich, St. Louis, MO) at various times of dark recovery.

Fifty-six mice received a course of 2, 4, or 8 days of intraperitoneal injections of minocycline (Sigma-Aldrich) at a dose of 45 mg/kg body weight or its vehicle (normal saline) twice daily for the first 4 days starting from 1 day before the exposure to light and once daily for the remaining 4 days for the groups maintained until 7 days after exposure. This dose was chosen in accordance with the study by Wu et al. showing no major adverse effects at 45 mg/kg body weight twice daily in a mouse model of Parkinson’s disease and the study by Arvin et al. showing a dose-dependent neuroprotective effect of minocycline with a maximum protection at 45 mg/kg in an animal model of transient ischemia. Additional control animals, not exposed to light, were either untreated (n = 4) or were given minocycline treatment (n = 5) for 8 days. The animals were euthanatized after 1, 3, or 7 days of dark recovery. Photoreceptor degeneration and microglial activation were evaluated respectively by morphology and morphometry of outer nuclear layer (ONL) thickness as well as by counting of CD11b+ microglial cell bodies in the ONL and subretinal space.

For morphologic study, the animals were euthanatized at 1, 3, or 7 days after exposure to light, and the eyes were collected and examined with light microscopy. For morphometric study (10 vehicle control mice, 8 minocycline-treated mice), the eyes were collected at 7 days after exposure to light. Two eyes of one animal were averaged as one sample for statistical analysis. The eyes were fixed in a mixed fixative (formalin, glacial acetic acid, and 70% alcohol with volume ratio 2:1:20) and cryosectioned. All eyes were cut vertically, and only sections through optic nerves were collected for the study. ONL thickness was measured at six locations of the retinal sections (superior quadrant: S1, S2, S3; inferior quadrant: I1, I2, I3; see Fig. 1A) starting from either side of the optic nerve with each segment at 0.5 mm apart with an image-analysis system (Microplan II; Laboratory Computer Systems, Inc., Cambridge, MA) at ×100 magnification. For CD11b immunolabeling of microglial cells (CD11b monoclonal antibody; 1:200 dilution; Serotec, Raleigh, NC), the animals (six vehicle control, six minocycline-treated) were euthanatized at 3 days after light-induced injury, and the eyes were fixed in 4% paraformaldehyde, processed, and embedded in Taab’s embedding medium for the avidin-biotin complex (ABC) kit (Vector Laboratories, Burlingame, CA) and visualized with 3,3′-diaminobenzidine (DAB). The immunohistochemical control experiments included a negative control and isotype control, using the specific IgG subtype. The microglial cell bodies were counted with a microscope at ×400 magnification, covering the whole retinal section extending from one ora serrata to the other through the optic nerve head. Student’s t-test and the Mann-Whitney rank sum test were used for statistical purposes to evaluate the preservation of photoreceptors and microglial inhibition by minocycline treatment.

An electoretinogram (ERG) was recorded according to a procedure adapted from a published rodent protocol in 14 mice (9 vehicle treated and 5 minocycline-treated) at 7 days after exposure to light. Briefly, before ERGs measurements animals were dark adapted overnight and anesthetized with a mixture of ketamine (75 mg/kg intramuscularly) and xylazine (13.6 mg/kg intramuscularly). Pupils were dilated, and a heating pad was used to keep the body temperature at 38°C. The cornel electrode was a gold wire loop; a differential electrode was placed on the forehead, and a neutral electrode was inserted subcutaneously near the tail. Responses were amplified at a gain of 10,000 between 0.1 and 1 kHz (~3 dB points), digitized at 5.12 kHz rate and computer averaged. Ganzfeld white light illumination was provided by a xenon visual stimulator with a flash duration of 10 μs (model PS22; Grass Telefactor, West Warwick, RI). Maximum intensity was 0.65 log cd·s·m−2 and attenuated over a 6-log range (step 1 log unit) with neutral-density filters (Eastman-Kodak, Rochester, NY). The flash was controlled by an electronic timer. In scotopic ERG recording, the intrastimulus interval (ISI) at low intensities was more than 10 seconds. At high intensities, the ISI was set greater than 20 seconds. In photopic ERG recording, a rod-function-suppressing Ganzfeld background of 30 cd/m2 was applied for 10 minutes. Luminance was calibrated with an integrating radiometer/photometer (model IL-1700; International Light, Newburyport, MA). Amplitudes of dark-adapted ERG a- and b-waves and light-adapted ERG b-waves were measured and averaged between the two eyes of the same animal as one value in each animal for statistical analysis. One-way ANOVA and Student’s t-test with Bonferroni correction were performed for comparing the minocycline-treated and light-damaged vehicle control groups at a given intensity with P < 0.05 deemed significant.

**Results**

**Morphologic and Morphometric Study**

Morphologically, at 1 day after exposure to light, mild disorganization of the outer and inner segments of the photoreceptor cells was noted in the vehicle-treated group, but they appeared unremarkable in the minocycline-treated retinas (not shown). At 3 days after exposure to light, the thickness of the ONL was mildly decreased in the vehicle-treated group, whereas it was better preserved in the minocycline-treated group (not shown). At 7 days after exposure to light, in the vehicle-treated group, the ONL was markedly thinned and the outer segments (OS) were shortened and disorganized. There was regional susceptibility, with the superior S2 region exhibiting the most severe damage (Fig. 1C). In contrast to the severe loss and damage of the photoreceptor cells of the vehicle-treated group, the ONL and OS of the minocycline-treated group were remarkably well preserved in all regions of the retina (Figs. 1D, 1F). The inner retinas were unremarkable in all light-exposed animals throughout the observation period.

Morphometric analysis showed that minocycline treatment provided highly significant preservation of photoreceptor cells after retinal photic injury in both the superior and the inferior quadrants of the retina (Table 1; Fig. 2). The overall average shows that the microglial-treated animals had 89.1% of the normal-appearing photoreceptor nuclei remaining versus 38.0% of the vehicle-control group (Table 1; Fig. 3). Minocycline treatment given to normal mice without exposure to light had no effect on the ONL thickness of the retinas (49.6 ± 2.5 μm).

In our previous study of this animal model (Zhang C, et al. *IOVS* 2003;43:ARVO E-Abstract 5122), we noted that there was a peak activation of microglia in the retina at 3 days after exposure to light. Therefore, in this study, we focused on microglial cell counts at that time point. Figure 4 shows CD11b immunolabeling in the superior S2 region of the retina at 3 days after exposure to light. In the normal retina, no CD11b+ cells were observed in the ONL and subretinal space (not shown). In the vehicle-treated group, many CD11b+ round or ameboid cells were seen in the ONL and subretinal space, whereas in the minocycline-treated group, there were only a few lightly stained CD11b+ ameboid cells in the outer retina. Counting of CD11b+ cells in whole retinal sections showed a highly significant reduction (Fig. 5; 63.5% reduction) in the minocycline-treated animals (17.5 ± 7.6) when compared with that of the light-exposed vehicle-control group (48.0 ± 6.0; P < 0.01).

**Electrophysiologic Study**

The effect of minocycline on light-induced retinal degeneration was further examined electrophysiologically. Activity of the
FIGURE 1. Photomicrographs of mouse retinas in light-induced photoreceptor degeneration (hematoxylin-eosin staining) at 7 days after exposure to light. (A, B) Low magnification of vertical sections through the optic nerve in the vehicle-treated (A) and minocycline-treated (B) retinas. S1, S2, and S3 indicated three measurements in the superior quadrant of the retina; I1, I2, and I3 indicated three measurements in the inferior quadrant. (C, D) High magnification showed remarkable thinning of ONL thickness with marked thinning and disorganization of the outer segments in the vehicle-treated retina (C), whereas the ONL and inner and outer segments were better preserved in the minocycline-treated retina (D) in the superior quadrants. (E, F) In the inferior quadrants, the ONL in the minocycline-treated retina (F) showed significantly better preservation than that in the vehicle-treated retina (E). ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer. Bars: (A, B) 200 μm; (C–F) 50 μm.

The rod system was examined with dark-adapted ERG at variable stimulation intensities (−1.35 to +0.65 log cd · s/m²), as shown in Figures 6A–C. Cone function was examined with light-adapted ERG (−0.35 to 0.65 log cd · s/m²) measurements (Figs. 6A, 6D).

With dark-adapted ERG, minocycline-treated eyes showed significantly higher responses in the rod photoreceptor a-wave amplitudes with stimulation intensities ranging from −0.35 to 0.65 log cd · s/m² (P < 0.05; Fig. 6B) when compared with the vehicle-treated light-damaged eyes. The difference in a-wave threshold between the two groups was less than 0.5 log cd · s/m² (20 μV criteria; Fig. 6B). At an intensity level of −1.35 log cd · s/m², there was no significant difference in the amplitudes of a-waves between the two groups. Based on the light stimuli used in this study, we divided the dark-adapted ERG b-wave into two segments: from −4.35 to −2.35 log cd · s/m², and from −1.35 to 0.65 log cd · s/m² (Fig. 6C). The former reflects the relatively uncontaminated rod-bipolar cell responses, whereas the latter contains signals generated from both rod- and cone-bipolar cells. In the high-intensity segment, the dark-adapted ERG b-wave amplitudes were significantly higher (P < 0.05 at −1.35 to −0.35 log cd · s/m², and P < 0.01 at 0.65 log cd · s/m²) in minocycline-treated eyes than in the vehicle-treated, light-damaged eyes. The difference in the b-wave threshold between the two groups was unremarkable (0.8 log cd · s/m², 50 μV criteria; Fig. 6C).

In light-adapted ERG, the a-wave was very small, and it may contain responses generated from the second-order OFF bipolar cells. Therefore, only b-wave was used to assess the cone function. Light injury greatly reduced the cone-driven b-wave, suggesting cone function was also severely affected. Minocycline treatment preserved the cone-driven signals significantly (P < 0.05; Figs. 6A, 6D).

DISCUSSION

In this study, we observed a remarkable neuroprotective effect against light-induced photoreceptor degeneration by intraperitoneal administration of minocycline, using morphologic, morphometric, and electrophysiological criteria. Concomitant with this highly effective neuroprotective effect, microglial responses as measured by cell counts of CD11⁺ cells in retinal

TABLE 1. Thickness of ONL in Superior and Inferior Quadrants of Mouse Retinas at 7 Days After Light-Induced Injury

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>I1</th>
<th>I2</th>
<th>I3</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>47.5 ± 4.6</td>
<td>48.8 ± 3.2</td>
<td>48.1 ± 4.3</td>
<td>46.8 ± 3.8</td>
<td>48.1 ± 2.4</td>
<td>46.8 ± 2.4</td>
<td>47.7 ± 2.4</td>
</tr>
<tr>
<td>Vehicle-treated</td>
<td>13.1 ± 8.0</td>
<td>9.5 ± 5.1</td>
<td>20.0 ± 10.4</td>
<td>14.5 ± 7.8</td>
<td>19.1 ± 8.6</td>
<td>32.8 ± 11.6</td>
<td>18.1 ± 7.7</td>
</tr>
<tr>
<td>Minocycline-treated</td>
<td>58.0 ± 8.7*</td>
<td>31.7 ± 10.3</td>
<td>40.8 ± 7.2</td>
<td>44.7 ± 9.0</td>
<td>49.2 ± 5.0</td>
<td>50.3 ± 2.3</td>
<td>42.5 ± 6.0</td>
</tr>
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The values are represented as mean micrometers ± SD. Normal, unexposed normal retinas; vehicle-treated, light exposed retinas with saline treatment; minocycline-treated, light-exposed retinas with minocycline treatment. S, superior; I, inferior.

* P < 0.01 when compared to the vehicle-treated retinas.
sections were also significantly reduced. These observations indicate that minocycline ameliorates photoreceptor degeneration and support our hypothesis that microglia/macrophages play a significant role in light-induced retinal degeneration.

A similar neuroprotective effect of minocycline has been observed in various animal models of neuronal degeneration such as the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson’s disease, and global and focal cerebral ischemia. In most of these studies, suppression of microglial/macrophagic responses was similar to this report. For example, in the MPTP-mouse model of Parkinson’s disease, minocycline was shown to protect nigral cells by inhibiting microglial activation resulting in a reduction in interleukin (IL)-1β and the inducible form of nitric oxide synthase (iNOS) levels. Similarly, Yrjanheikki et al. showed that minocycline reduced inflammation, protected neurons within a wide time window, and inhibited IL-1β converting enzyme (ICE) and cyclooxygenase (COX)-2. Other studies showed potent protective effects of minocycline in models of CNS degenerative diseases including multiple sclerosis and amyotrophic lateral sclerosis (ALS). Also pointed to a mechanism of inhibiting microglial cell activation. In a mixed spinal cord culture, minocycline prevented proliferation and activation of microglial cells before neuronal death and diminished the increased release of IL-1β and nitric oxide (NO).

Also inhibited the N-methyl-D-aspartate (NMDA)-induced activation of p38 MAPK in microglial cells. Tetracycline, an analogue of minocycline, has also been shown to inhibit iNOS mRNA expression in murine macrophages. Our present results are consistent with findings in these earlier studies and extend our understanding to retinal photoreceptors. In contrast, recent studies have also demonstrated that minocycline prevents neuronal loss by direct inhibition of cytochrome c release and by inhibiting caspase-dependent/independent cell death pathways. Therefore, it is possible that neuroprotection of photoreceptors by minocycline in this study is a combination of diminished microglial/macrophagic activation and a direct action on cell death pathways. Further studies are needed to delineate its mechanism(s) of action.

Minocycline has been used in other CNS studies with doses between 11.25 and 45 mg/kg twice daily. A pharmacokinetic study in rats by Colovic and Caccia showed that minocycline readily crosses the blood–brain barrier, achieving mean brain concentrations at 30% to 40% of the plasma concentration, regardless of the dose and route of administration. Wells et al. reported a serum level of 5 to 10 µg/mL, using a regimen of minocycline administration similar to ours. Using the serum measurements of Wells et al. and the findings of Colovic and Caccia, minocycline concentration in brain tissue and probably in retinal tissue could be estimated to be in the range of 1.75 to 3.5 µg/mL corresponding to 3.5 to 7 µM. Tikka and Koistinaho reported that minocycline provides neuroprotection against NMDA neurotoxicity by inhibiting microglia at a concentration as low as 0.2 µM in a mixed spinal cord culture system. Zhu et al. and Wang et al. showed that minocycline inhibits the release of cytochrome c or caspase-dependent and -independent cell death pathways, respectively, at a dose of 10 mg/kg per day. The dose we used in this study (90 mg/kg per day) would probably result in a minocycline tissue concentration higher than those in these three reported studies. Therefore, it is possible that minocycline at the dose used in our study would work through all these pathways. The exact mechanism(s) of minocycline’s neuroprotective effect and the role of microglial/macrophagic responses requires further study.

Consistent with previous studies, retinal light damage was predominantly found in the outer retina. Of particular interest is our ERG results, which showed that both dark- and light-adapted ERG waveforms were reduced more than 80% at the highest stimulation intensity (0.65 log cd · s/m²), suggesting that light insults severely reduce rod and cone functions. In
contrast, in other retinal degeneration models, cone function was better preserved than rod function at early stages. This discrepancy in cone response between this study and other models may occur because green light was used to inflict the damage in our model. Two types of cones are known to exist in the mouse retina. The peak sensitivity of the ultraviolet (UV)-cone is approximately 360 nm and the middle-wavelength (M)-cone is near 512 nm. And the M-cones were
found to comprise 85–95% of cones.\textsuperscript{40,41} In addition, whereas the rods (500 nm) are uniformly distributed, the two types of cones are unevenly distributed in the mouse retina, with most M-cones located in the superior hemisphere and the UV-cones located at the inferior hemisphere.\textsuperscript{43} Because of an overlap of the action spectra, the green light used (490–580 nm, peak at 535 nm) may inflict more severe damage on the predominant M-cones (as well as the rods) and thus reduce the cone-driven function. This reasoning is also supported by our morphologic and morphometric findings that the superior retina sustained more severe damage than the inferior quadrants.

Unlike the remarkable loss of photoreceptors, the inner retina appeared less affected by the light insults morphologically. This property mimics that of other retinal photoreceptor degeneration models.\textsuperscript{38,42} Although postreceptoral ERG signals in the damaged retinas were also decreased, the reduction could be attributable to the loss of photoreceptors and a decrease of phototransduction signals rather than direct damage to bipolar cells and other inner retinal neurons. This explanation is supported by histologic findings that the ONL was severely damaged, whereas the inner retinal structures were well preserved. Furthermore, when photoreceptors were preserved by minocycline treatment, the inner retina ERG components b-wave and the oscillatory potentials recovered proportionately to the photoreceptor a-wave. Hence, the green light mainly caused rod and M-cone damage. The ERG findings also support the observation that the neuroprotective effect of minocycline in retinal light damage was expressed in the photoreceptor layer preserving both rod and cone function.

In many neuronal degenerative diseases of the brain such as multiple sclerosis and Parkinson’s and Alzheimer’s diseases, microglial cells were activated and recruited by the degenerating neurons.\textsuperscript{1,2,43,44} These activated microglial cells remove the degenerating neurons, and at the same time secrete many proinflammatory cytokines, which may be cytotoxic to neurons. This is supported by the studies demonstrating that inhibition of microglial activation or decrease of the synthesis and secretion of microglial products protects neurons against neurodegenerative changes.\textsuperscript{13–15,45,46} and by studies showing the involvement of chemokines in neuronal degenerative, ischemic, and inflammatory diseases in the brain.\textsuperscript{47–50} In the eye, investigators have demonstrated that microglial cells from the inner retina migrate into the ONL and subretinal space in the photoreceptor degeneration in light-exposed animals and the retinal dystrophy of RCS rats,\textsuperscript{5,7–9,31} suggesting a possible involvement of activated microglia in photoreceptor degeneration. This study of amelioration of light-induced retinal degeneration by minocycline, a compound known to inhibit microglial activation, further supports an important role of microglia in light-induced retinal degeneration. Activated microglial cells in the outer retina may secrete various toxic cytokines and exaggerate photoreceptor cell damage in light-induced retinal degeneration. As a result, inhibition of microglia may allow more photoreceptor cells to survive this insult.

Minocycline is a semisynthetic second-generation tetracycline and an antimicrobial drug. It is also a potent drug for immunomodulatory and anti-inflammatory actions.\textsuperscript{52} Recent studies have shown that its anti-inflammatory effects were unrelated to its antimicrobial actions.\textsuperscript{53–55} Minocycline also inhibits matrix metalloproteinases, which play an important role in the inflammatory process.\textsuperscript{27} Minocycline is absorbed rapidly from the gastrointestinal tract with a half-life of 18 hours. It is highly lipid soluble and has a superb penetration of the blood–brain barrier. Clinical trials of minocycline for rheumatoid arthritis have shown its safety and efficacy;\textsuperscript{51} but adverse gastrointestinal effects, hyperpigmentation, and intracranial hypertension were noted in some patients. In animal studies by Yrjanheikki et al.\textsuperscript{16} and Wu et al.\textsuperscript{15} no significant adverse effects were observed with a dose of minocycline of 90 mg/kg per day. In our experiment, we noticed an approximately 15% weight loss in mice after an 8-day minocycline treatment. This observation is consistent with the study of Lee et al.\textsuperscript{57} Considering its remarkable neuroprotective effects against light-induced photoreceptor damage, minocycline may be a good candidate for a novel therapy in human photoreceptor degenerative diseases by inhibiting activation of retinal microglial cells after pharmacodynamic and toxicologic factors have been taken into account.

In summary, minocycline provided marked neuroprotection to the light-damaged photoreceptor cells, probably through the inhibition of microglia. Because of its pharmacokinetic properties and its desirable pharmacologic actions such as anti-inflammation, neuroprotection, and inhibition of endothelial cell proliferation (thus antiangiogenesis)\textsuperscript{54} and its proven safety in clinical use, minocycline may be a good candidate for treatment of photoreceptor degenerative diseases. Currently, minocycline is under study in a clinical trial for Parkinson’s disease, and it is hoped that it will be considered for the clinical treatment of retinitis pigmentosa or the dry form of age-related macular degeneration, both of which involve photoreceptor degeneration.

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