Investigating the Effect of Ciliary Body Photodynamic Therapy in a Glaucoma Mouse Model

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PURPOSE. To investigate the morphologic and functional effects of verteporfin ciliary body photodynamic therapy (PDT) in a murine glaucoma model and normal mouse eyes.

METHODS. A glaucomatous mouse strain, DBA/2J and a normal control mouse strain (C57BL/6) were used in the study. Verteporfin was injected intravenously at doses of 1.0 (DBA/2J) or 2.0 or 4.0 (C57BL/6) mg/kg. Transscleral irradiation of the ciliary body was performed with light at a wavelength of 689 nm delivered through an optical fiber, with irradiance of 1800 mW/cm² and fluence of 100 J/cm². Laser irradiation was applied for 360° of the corneoscleral limbus in C57BL/6 normal mice and for 180° in DBA/2J mice. Retreatment was performed in C57BL/6 normal mice that had been treated with 2.0 mg/kg of verteporfin at post-PDT day 7. One eye of each animal was treated, and the fellow eye served as the control. The morphologic effect of PDT on the ocular structures was assessed by light and electron microscopy. The IOP was measured using an applation tonometer with a fiber-optic pressure sensor. Surviving retinal ganglion cells (RGCs) in DBA/2J mice eyes were retrogradely labeled with a neurotracer dye at 12 weeks after PDT.

RESULTS. In all groups, almost all ciliary body blood vessels in the treated area were thrombosed 1 day after PDT. In DBA/2J mice, ciliary epithelium and stroma were severely damaged 1 day after PDT. The mean IOP in treated eyes was significantly reduced compared with that in the control eyes in all groups. The reduction of mean IOP in DBA/2J mouse eyes persisted for 7 weeks, although the mean IOP in normal mouse eyes treated with 2.0 or 4.0 mg/kg verteporfin returned to the level of the fellow control eyes by 7 and 17 days after treatment, respectively. The mean number of RGCs in the DBA/2J treated eyes was significantly higher than in control eyes.

CONCLUSIONS. Ciliary body PDT resulted in morphologic changes in the ciliary body, significant reduction of IOP, and prevention of ganglion cell loss in a mouse glaucoma model. These results suggest that ciliary body PDT is a more selective cyclodestructive technique with potential clinical application in the treatment of glaucoma.

Glaucoma is the most common cause of blindness and the second leading cause of irreversible blindness among African Americans in the United States.1 It is also the leading cause of blindness among U.S. Hispanics.1–2 Lowering intraocular pressure (IOP) using medical or surgical therapy is the mainstay of therapy, designed to control and limit this common disease. However, treating glaucoma remains a difficult task, since most medications have side effects, lose their efficacy, and require patients’ life-long compliance. Surgical methods have a higher risk of complication. Ciliary body destruction by cryotherapy or laser irradiation represents a useful alternative for the management of glaucoma that is resistant to other modes of therapy.3–5 However, the current cyclodestructive techniques have a high rate of side effects including loss of vision, hypotony, macular edema, or phthisis bulbi.6–8

Photodynamic ciliary body destruction has been reported to be a better cyclodestructive technique, with potential clinical application in the treatment of glaucoma.9–12 Previous studies of transscleral ciliary body photodynamic therapy (PDT) in normal rabbit eyes using the photosensitizer chloraluminum sulfonated phthalocyanine (CASPCe) have shown a decrease in IOP for 2 weeks after treatment.12,13 In this study, we used liposomal benzoporphyrin derivative (verteporfin) as a photosensitizer, because it is a clinically approved and well-tolerated PDT drug and is also a suitable drug for ciliary body PDT. Verteporfin is the first light-activated drug approved by regulatory authorities for the treatment of choroidal neovascularization secondary to age-related macular degeneration.11 It is a synthetic chlorin-like porphyrin that is activated by low-intensity, nonthermal, laser light at a wavelength of ~690 nm.14,15 It is prepared as a liposome-based formulation, associates with serum lipoproteins including low-density lipoproteins (LDL), and is thought to be taken up preferentially by cells expressing high levels of LDL receptors, including neovascular endothelial cells and tumor cells.16–19 Zheng et al.20 have investigated the distribution of LDL receptors in rats, specifically using immunohistochemistry to localize gp330 and the LDL receptor–related protein (LRP/α2MR). They reported that ciliary and retinal epithelial cells were the only cells that expressed both LRP/α2MR and gp330, suggesting that ciliary body epithelium would be a target for verteporfin.

To our knowledge, no studies of ciliary body PDT have been performed in a glaucoma model, and treatment effects in normal eyes have been short in duration. It is important to test PDT in a model with elevated IOP, to determine whether PDT cyclodestruction may be a useful therapy in glaucoma. The strain of DBA/2 mice was established in the 1930s and has a predisposition to development of various tumors, calcific heart lesions, gastritis, duodenal polyps, calcareous pericarditis, and degenerative processes of the myocardium, skeletal muscle, subcutaneous adipose tissue, and blood vessels.21 Abnormalities in the anterior segment of the eye in this strain were first reported in 1986.22 Shortly thereafter, it was reported that DBA/2Nia mice exhibit peripheral anterior synecchia, iris

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Supported by the Massachusetts Lions Eye Research Fund and National Eye Institute Grant EY014104.

Submitted for publication July 23, 2005; revised December 28, 2005; accepted March 31, 2006.

Disclosure: A. Matsubara, None; T. Nakazawa, None; D. Husain, None; E. Iliaki, None; E. Connolly, None; N.A. Michaud, None; E.S. Gragoudas, None; J.W. Miller, None

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atrophy, pigment dispersion, depletion of retinal ganglion cells (RGCs), and degeneration of the optic nerve. In 1998, similar changes were reported in DBA/2J mice, another DBA sub-strain. Mice of this strain have spontaneous development of essential iris atrophy, pigment dispersion, and glaucomatous changes. The IOP was elevated in most mice by the age of 9 months, followed by ganglion cell loss, optic nerve atrophy, and optic nerve cupping. The mean IOP of eyes of 6-month-old females was significantly higher than that of the eyes of males of similar age, and this sex difference in IOP was still evident at the age of 9 months. As glaucoma developed, the ganglion cell loss and mild cupping of the optic nerve were present in some animals by 11 months and in most of the mice by the age of 22 months.

In this study, we used a liposomal benzoporphyrin derivative (verteporfin) as a photosensitizer and used female DBA/2J mice aged 8 months as a glaucoma mouse model to investigate the effect of ciliary body PDT on IOP and glaucomatous morphologic changes. We also used a normal mouse strain (C57BL/6) to evaluate the cyclodestructive effect of ciliary body PDT based on morphologic and functional alterations.

**Materials and Methods**

**Animal Model**

All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research using a protocol approved by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. C57BL/6 female mice aged 3 months (n = 20) were used as a normal control, and DBA/2J female mice aged 8 months (n = 20) were used as a glaucoma mouse model. The number and type of animals for each phase of the study is shown in Table 1. One eye of each animal was treated, and the fellow eye served as the control. Animals were housed in covered cages, fed with a standard rodent diet ad libitum, and kept in a constant 12-hour light-dark cycle. For general anesthesia, a mixture of ketamine (100 mg/kg) and xylazine (9 mg/kg; both from Phoenix Scientific, Inc., St. Joseph, MO) was administered intraperitoneally.

**Photodynamic Therapy**

The photosensitizer, verteporfin (Novartis Ophthalmics, Inc., Duluth, GA) was used and prepared according to the manufacturer’s instructions. Anesthetized mice received the photosensitizer drug by tail vein injection with a 30-gauge needle 5 minutes before laser irradiation. A verteporfin dose of 2.0 or 4.0 mg/kg was used in C57BL/6 normal mice, and 1.0 mg/kg was used in DBA/2J mice (total volume 0.25–0.4 mL on the basis of weight). The laser irradiation to the ciliary body was applied transscerally using a 600-µm optical fiber at a wavelength of light of 689 nm, with a diode laser (Lumenis, Santa Clara, CA), with irradiance 1800 mW/cm² and fluence of 100 J/cm² (measured at the sclera). Transsceral irradiation of the ciliary body was performed with applications for 180° of the corneoscleral limbus in C57BL/6 normal mice and for 180° in DBA/2J mice. Sixteen and eight spots, respectively, were applied to overlap each other (Table 2). Each treatment spot took 55 seconds and therefore it took 16 or 8 minutes to complete the laser irradiation. However, in the eyes prepared for lectin labeling and terminal deoxynucleotide transferase-mediated nick end labeling (TUNEL), PDT was performed in C57BL/6 mice to cover 180° of the ciliary body, using the 4.0-mg/kg verteporfin dose to compare the degree of staining between the irradiated and nonirradiated areas.

Retreatment was performed in C57BL/6 normal mice, which had been treated with 2.0 mg/kg of verteporfin after the IOP measurement at post-PDT day 7, with the same light parameters. In this study we used a lower dose of verteporfin and smaller area of irradiation in the DBA/2J mice, because preliminary experiments using 2.0 mg/kg in DBA/2J mice and irradiation for 360° of the corneoscleral limbus resulted in severe damage, with corneal edema, hyphema, and corneal neovascularization in 9 of 10 eyes. After the reduction of the extent of treatment by half, these complications were still seen in two eyes of three. After we reduced both drug dose and extent of treatment by half, these complications did not develop.

**IOP Measurement**

The IOP was measured with a previously reported applanation tonometer (Ahamed E, et al. IOVS 2003;44:ARVO E Abstract 3356)25 (n = 12 in DBA/2J mice, n = 5 in C57BL/6 mice in each dose). The applanation tonometer employs a fiber-optic pressure sensor that is designed
samples were embedded in optimal cutting temperature (OCT) compound overnight and were immersed in graded sucrose in 0.1 PBS. The enucleated eyes were fixed in 4% paraformaldehyde in 0.1 PBS (pH 7.4) was perfused after PBS, to remove residual unbound lectin. Enucleated eyes were fixed in 4% paraformaldehyde in 0.1 PBS overnight and were immersed in graded sucrose in 0.1 PBS. The samples were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Tokyo, Japan) and sectioned vertically at 10 μm.

**Histology**

Eyes for light and transmission electron microscopic examination were enucleated at 6 hours (DBA/2J only), 1 and 7 days after treatment and at the end of IOP follow-up (8 weeks after treatment; n = 2 in each group at each time point). The eyes were bisected behind the limbus and placed in modified Karnovsky's fixative at 4°C overnight and then transferred to 0.1 M cacodylate buffer at pH 7.4. Ciliary body specimens in normal mouse eyes in a 4.0-mg/kg dose group were observed before postfixation by stereomicroscopy at 1 day after PDT. Tissues were postfixed in aqueous 2% osmium tetroxide, stained en bloc with uranyl acetate, dehydrated in graded ethanolos, and embedded in Epon. One-micrometer-thick sections were stained with 0.5% toluidine blue in borate buffer for light microscopy and examined with a photomicroscope (Leica Microsystems, Wetzlar, Germany). Thin sections were stained with aqueous uranyl acetate and Sato's lead stain, and examined with a transmission electron microscope (CM 10; Phillips, Eindhoven, The Netherlands).

**Lectin Labeling of Vascular Endothelial Cells and TUNEL Staining**

Lectin labeling of vascular endothelial cells and TUNEL staining was performed 1 day after PDT in C57BL/6 normal mouse eyes (n = 2). In DBA/2J mice, only TUNEL staining was performed (n = 2). PDT was performed to cover 180° of the ciliary body using 4.0 (C57BL/6) and 1.0 (DBA/2J) mg/kg verteporfin to compare the degree of staining between the irradiated and nonirradiated areas. Non-surgically treated fellow eyes (n = 2) were used as the control. Anesthetized animals were perfused with 8 mL phosphate-buffered saline (PBS) administered via a catheter in the left ventricle. After PBS perfusion, fluorescein-isothiocyanate (FITC)-coupled concanavalin A lectin (20 μg/mL in PBS [pH 7.4]); 5 mg/kg BW; Vector Laboratories, Burlingame, CA) was perfused to stain vascular endothelial cells. Paraformaldehyde (4%) in 0.1 PBS (pH 7-4) was perfused after PBS, to remove residual unbound lectin. Enucleated eyes were fixed in 4% paraformaldehyde in 0.1 PBS overnight and were immersed in graded sucrose in 0.1 PBS. The samples were embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Tokyo, Japan) and sectioned vertically at 10 μm.

**Figure 1.** Left: control eye; right: treated eye, showing pale, swollen ciliary processes (arrow) after PDT.

TUNEL staining was performed according to the protocol of the manufacturer (Fluorescein In Situ Apoptosis Detection Kit; Chemicon International, Temecula, CA) to detect retinal cell death induced by PDT. Sections were counterstained with 1 μg/mL DAPI (Sigma-Aldrich, St. Louis, MO).

**RGC Count in DBA/2J Mice**

Four weeks after the end of IOP follow-up, RGCs were counted in the DBA/2J mouse eyes (11 months old), as previously reported,27,28 with a slight modification (n = 4). In mice under anesthesia, the skin over the cranium was incised and the scalp exposed. Holes approximately 2 mm in diameter were drilled in the skull 4 mm posterior to the bregma and 1 mm lateral to the midline on both sides of the midline raphe. A neurotracer dye (4% solution in saline; Fluorogold; Fluorochrome, Englewood, CO) was directly applied (1 μL, at a rate of 0.5 μL/min) at a depth of 2 mm from the brain’s surface through a syringe (Hamilton, Reno, NV). Skull openings were then sealed with antibiotic ointment. The overlying skin was sutured and antibiotic ointment applied externally.

Seven days after the application of the fluororescent tracer, the eyes were enucleated and the retinas were dissected, fixed in 4% paraformaldehyde (PFA) and flatmounted on glass slides. Cell counting was performed as previously described27 under a fluorescence microscope (Leica Microsystems) using a UV filter set. RGC densities were determined by counting the tracer-labeled RGCs in 12 distinct areas of 9.0 × 10⁻⁷ mm² each (three areas per retinal quadrant at one sixth, one half, and five sixths of the retinal radius). The density of fluorochrome-labeled RGCs was defined as the average number of cells in the 12 fields. Cell counting was performed in a masked fashion.

**Statistical Analysis**

All data are presented as the mean ± SD. A paired group of two were compared by paired ttest. To compare three groups, data were compared by ANOVA, with post hoc comparisons tested using the Bonferroni procedure. P < 0.05 was considered to be statistically significant.

**Results**

**Histology**

**Stereomicroscopic Findings.** Ciliary processes appeared pale and swollen compared with the control eyes in C57BL/6 normal mice 1 day after PDT with the 4.0-mg/kg dose (Fig. 1).

**Light and Electron Microscopic Findings.** All ciliary body vessels in C57BL/6 normal mouse eyes were open...
in control eyes (Fig. 2A). Electron microscopy showed patent capillaries surrounded by pigmented and nonpigmented epithelium (Fig. 2B). The basal plasma membrane of the pigmented epithelium had marked infolding, indicating that the cells were actively involved in ion transport (Fig. 2C). One day after PDT, the ciliary processes in normal mouse eyes treated with 2.0 mg/kg verteporfin were enlarged due to edema, compared with the control (Figs. 2A, 2D). Most ciliary body blood vessels appeared thrombosed (Fig 2D). Iris changes or anterior chamber reactions were not seen in C57BL/6 mice eyes. The same findings were observed in normal mouse eyes treated with 4.0 mg/kg verteporfin. The significant morphologic changes in the ciliary body included vascular injury and thrombosis, with vacuolization of endothelial cells and extravasation of leukocytes and erythrocytes. The basal infoldings were abnormally separated due to edema (Fig. 2E). The basal processes of the pigment epithelium around the vessels were distended. Cell nuclei and mitochondria in both layers appeared unchanged. The ciliary processes appeared normal (toluidine blue) (Fig. 2F). Basal processes of the pigmented epithelium were again abundant, although they appeared more compact (Fig. 2I). Cytoplasmic and nuclear morphology were normal. PE, pigmented epithelium; NPE, nonpigmented epithelium; L, leukocyte; Er, erythrocyte; V, vessel. Scale bars: (B) 5 μm; (C, F, I) 2 μm; (E, H) 10 μm. Magnification: (A, D, G) ×50.

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thelial cells were edematous, and some epithelial cells were disrupted. Organelle dissolution and rupture of the plasma membrane were seen (Fig 3B). One day after PDT, most ciliary body blood vessels were still thrombosed, and the ciliary epithelium and stroma were severely damaged and edematous. Large intercellular spaces were occupied by exudates. Electron microscopy showed that basal infoldings were stretched and swollen with exudate. Epithelial edema and abnormal intercellular fluid were noted (Figs. 3C, 3D). At 7 days after PDT, the edema was decreasing in the epithelial layers. Some of the ciliary body blood vessels were still thrombosed, and some ciliary epithelia still showed edematous swelling. Electron microscopic findings showed that the basal infoldings were still separated due to edema. Some ciliary blood vessels were recanalized and endothelia were normally organized. Possible regenerative epithelia were seen, and vacuolations were still visible in the epithelial layer (Fig. 3F). At 8 weeks after PDT, ciliary body blood vessels were recanalized, and edema had disappeared. Some ciliary processes were small and flattened. However, it was difficult to tell the difference between the PDT-treated and the untreated ciliary body, because even some of the untreated ciliary processes showed atrophic changes at this age. We could not detect any abnormality, including retinal detachment, in the peripheral retina in the PDT-treated area.

Lectin Labeling of Vascular Endothelial Cells and TUNEL Staining

A section through the untreated and treated areas of the ciliary body in C57BL/6 normal mice 1 day after PDT with 4.0 mg/kg verteporfin are shown in Figures 4A and 4B, respectively. The vessels in the ciliary body and sclera on the untreated side showed abundant staining by lectin (green fluorescence), and no TUNEL-positive cells (red fluorescence) were observed (Fig. 4A). In contrast, there were some TUNEL-positive cells in the ciliary body and retina on the PDT-treated side. The vessels in the ciliary body on the treated side showed very few cells stained by lectin (green fluorescence) because of thrombosis (Fig. 4B). In DBA/2J mouse eyes, only TUNEL staining was performed 1 day after PDT. Many TUNEL-positive cells were seen in the ciliary processes in both the treated and untreated regions of the treated eye, whereas no TUNEL-positive cells were noted in the nontreated eye (Figs. 4C-E). During the laser irradiation, we found that the eyes of the DBA/2J mice were readily transilluminated compared with the C57BL/6, probably due to loss of pigment caused by pigment dispersion. In other
words, in DBA/2J mice, the retina-choroid on the side opposite the laser received light irradiation, probably due to these pigment abnormalities. A few TUNEL-positive cells were seen in the peripheral retina in treated region, but there are no TUNEL-positive cells in the retina and choroid in the untreated region. TUNEL-positive cells in the untreated side of the treated eye were located only within the ciliary processes, suggesting that laser light reached the opposite untreated region.

IOP Measurement

In normal mouse eyes treated with 2.0 mg/kg verteporfin, the mean baseline IOP was $13.2 \pm 2.4$ mm Hg in the left eye and $13.6 \pm 2.1$ mm Hg in the right eye (Fig. 5A). There was no significant difference between the baseline measurements in control and treated eyes. The mean IOP in treated eyes was significantly reduced at 1 and 3 days after treatment compared with the control eyes (by $35.1\%, P < 0.05$, and by $41.3\%, P < 0.01$, respectively). The mean IOP in the 2.0-mg/kg verteporfin group returned to the level of the fellow control eyes by 7 days after treatment. However, retreatment at 7 days after the first PDT significantly reduced the IOP again. The mean IOP in treated eyes was $8.7 \pm 2.6$ and $9.5 \pm 2.1$ mm Hg at 10 and 14 days after the first PDT, respectively. The mean IOP returned to the level in the fellow control eyes by 17 days after PDT. The mean IOP in treated eyes was significantly lower than the level in fellow control eyes at 28 and 42 days after PDT.

In normal mouse eyes treated with 4.0 mg/kg verteporfin, the mean baseline IOP was $14.8 \pm 1.4$ mm Hg in the left eye and $14.2 \pm 0.9$ mm Hg in the right eye (Fig. 5B). There was no significant difference between the baseline measurements in control and treated eyes. The mean IOP of treated eyes was significantly reduced ($9.2 \pm 2.4$ mm Hg, $P < 0.05$) at 1 day after treatment and reached a minimum ($7.2 \pm 1.9$ mm Hg, $P < 0.01$) at 5 days after treatment compared with the control eyes, by $42.1\%$ and $47.3\%$, respectively. Significant reduction of mean IOP lasted for 14 days and returned to the level of the fellow control eyes by 17 days after treatment.

In DBA/2J glaucomatous mice, the mean baseline IOP was $18.8 \pm 1.9$ mm Hg in the left eye and $18.8 \pm 4.3$ mm Hg in the right eye at 8 months of age (Fig. 5C). The mean IOP 1 day after treatment was $18.2 \pm 6.5$ and $15.9 \pm 5.7$ in fellow control eyes and treated eyes, respectively, and there was no significant difference between the two groups 1 day after PDT. The mean IOP of treated eyes was significantly reduced ($9.2 \pm 2.4$ mm Hg, $P < 0.05$) at 1 day after treatment compared with the control eyes, by $42.1\%$ and $47.3\%$, respectively. Significant reduction of mean IOP lasted for 14 days and returned to the level of the fellow control eyes by 17 days after treatment.

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IOP at 4 (9 months old) and 8 (10 months old) weeks after PDT were 15.6 ± 3.7 and 12.6 ± 4.7 mm Hg, respectively (Fig. 5C, D). Although there was no significant difference between the mean IOP at 8 and 9 months of age, the mean IOP at 10 months of age was significantly lower than that at 8 months of age (P < 0.01; Fig. 5D).

**RGC Count in DBA/2J Mice**

RGCs stained by the gold neurotracer in the eyes of DBA/2J mice at 11 months of age are shown in Figure 6. The number of ganglion cells in DBA/2J control and treated eyes were 2701 ± 798 and 3905 ± 627/mm², respectively. The loss of RGCs over the 3-month course of follow-up was significantly reduced in mice receiving PDT compared with the DBA/2J control (P < 0.05; Fig. 7).

**DISCUSSION**

The notable morphologic changes after PDT of the ciliary body were vascular injury, thrombosis, and severe edema. Lectin staining confirmed the lack of perfusion of ciliary body blood vessels after PDT. Once verteporfin is activated by light in the presence of oxygen, highly reactive, short-lived singlet oxygen, and reactive oxygen radicals are generated, causing damage to the targeted cells. Verteporfin PDT has been shown to injure endothelial cells, resulting in vessel occlusion. Damaged endothelium is known to release von Willebrand factor, eicosanoids such as thromboxane and prostacyclin through the cyclo-oxygenase pathway, and leukotrienes through the lipoxygenase pathway. PDT also causes changes in the cytoskeleton and cell shape, which can lead to the interruption...
of the tight junctions between the endothelial cells, and causes exposure of parts of subendothelial connective tissue. Von Willebrand factor and other clotting factors such as thromboxane are released at the exposed site, which leads to platelet activation. The activated platelets stick to the gaps between the damaged endothelial cells, and further aggregation of platelets starts to occlude the vessel. In addition, eicosanoids such as prostacyclin induce increased vascular permeability and leakage.

In this study ciliary body PDT significantly reduced the IOP in both normal and glaucomatous eyes. In normal mouse eyes, we were able to demonstrate a dose-dependent effect of the drug, with a higher dose resulting in a longer period of IOP reduction in normal C57BL/6 mice. In contrast, both drug doses resulted in similar histologic findings at 1 and 7 days after PDT. We have previously reported that lowering the dye dose in both normal and glaucomatous eyes that lasted only 7 to 17 days. The strategy of ciliary body PDT may damage the ciliary body epithelium, which produces aqueous humor. In DBA/2J mice, we found not only vascular thrombosis but also ciliary body epithelial injury in histologic examination. In other words, we could get effective IOP reduction with less laser power and a smaller area of treatment. One explanation is that pigment may play a protective role against PDT and the more pigmented C57BL/6 mice are more resistant to treatment. Tsilimbaris et al. reported that the IOP reduction in pigmented rabbits by ciliary body PDT with CASPc lasted a relatively shorter period than that in albino rabbits. Because DBA/2J mice have pigment dispersion, there may be a decrease in pigment in the ciliary body. Experimentally, we found that the eyes of the DBA/2J mice were readily transilluminated during treatment compared with those in the C57BL/6 mice, which have a more pigmented eye. In light and electron microscopic findings, ciliary body vessel occlusions were visible, even in the untreated side of treated eyes (data not shown). TUNEL staining also suggested that the untreated side had been irradiated and damaged to some extent. Although pigmentation augments-laser induced cyclodestruction, in PDT, it can act as a protective barrier for the target tissue, preventing its exposure to light during photosensitizer circulation. There are some reports of the relationship between intensity or extent of cyclophotocoagulation and IOP reduction in the rabbit eye. van der Zypen et al. reported that the eye with normal perfusion pressure requires extensive vascular irradiation damage, and more than 50% of the total secretory surface must be destroyed before effective and long-term reduction in IOP is attained. In our preliminary experiments, we irradiated 180° of the ciliary body in normal mouse eyes, using 2.0 mg/kg verteporfin, but we did not get a significant IOP reduction. In contrast PDT with 1.0 mg/kg verteporfin and treatment of 180° resulted in a significant reduction of IOP in the DBA/2J mice. By the age of 11 to 15 months, the ciliary processes in DBA/2J mice are usually fewer, shorter, and narrower than those in young DBA/2J mice. The ciliary body epithelium of DBA/2J mice at age 8 to 10 months may have already started to decrease in size and number and therefore may not be able to compensate for the injury induced by PDT.

In this study, we used a lower dose of verteporfin and smaller area of irradiation in the DBA/2J mice. Preliminary experiments using 2.0 mg/mL of verteporfin in DBA/2J mice and irradiation for 360° of the corneoscleral limbus resulted in severe damage including corneal edema, hyphema, and corneal neovascularization. As mentioned, one reason for this increased sensitivity to PDT may be the loss of pigmentation that augments the destructive effect of PDT. Another reason may be the breakdown of the blood–ocular barrier in DBA/2J mice. Mo et al. reported that DBA/2J mice experienced breakdown of the blood–ocular barrier from 4 months of age and an inflammatory response was sustained for several months. They also reported that fluorescein leakage was observed in all eyes by 7 months of age, entering the anterior chamber from behind the iris and from the iris stroma. These findings suggest that DBA/2J mice may be susceptible to inflammation and thus to more pronounced damage from PDT. DBA/2J mouse eyes exhibit a progressive form of secondary angle-closure glaucoma that leads to ganglion cell loss in a time-dependent fashion. Using retrogradely labeled RGCs, Schuettauf et al. reported that significant age-dependent loss of RGCs is observed in 6- and 9-month-old DBA/2J mice and time-dependent loss of RGCs can be inhibited by conventional anti-glaucomatous therapy with β-blockers at 9 months of age. In contrast, using cell counts of histologic sections, John et al. reported that the number of surviving RGCs is normal by 8 to 9 months of age and in 5 of 16 eyes decreased by 33% to 40% by 11 to 15 months of age. In our study the age-dependent loss of RGCs between 8 and 11 months of age was significantly reduced in mice receiving PDT compared with the DBA/2J control. In this experiment we used only female DBA/2J mice, which are known to have higher IOPs than those of the male.
at 6 to 9 months of age. This selection may have increased the likelihood of finding a significant difference in treated and untreated eyes.

We detected a few, TUNEL-positive cells in the peripheral retina of the direct laser-irradiated area. Because the size of the laser probe was slightly large for the mouse eye, irradiation of some peripheral retina was unavoidable. However, histologic analysis after 2 months of follow-up showed no abnormality in the peripheral retina. These data suggest that the retinal PDT injury was not severe. Further development of the probe for treatment of the ciliary body, will reduce retinal damage to a minimum. In this study we used a 600-μm optical fiber and irradiated 8 or 16 spots during 8 to 16 minutes. In future we will examine ciliary body PDT in a bigger eye or in humans. As the length of human ciliary process is approximately 2000 μm, we will have to use a larger fiber in human treatment. Thus, the number of laser spots in human may be almost the same as in the mouse. Because pigment can act as a protective barrier for the target tissue in PDT, we have to give careful consideration to that point in future human experiments. Further considerations on not only laser parameters but also probe size are needed for clinical application. Additional studies comparing PDT and transscleral cyclophotocoagulation with diode or Nd:YAG laser would be of interest.

The most significant advantage of ciliary body PDT compared with conventional laser cyclodestruction techniques is the less-destructive nature of the lesions induced by PDT. Moreover, we were able to achieve significant and more prolonged IOP reduction as well as prevention of ganglion cell loss in glaucomatous DBA/2J mouse eyes in this study. Although further investigations are needed to refine treatment parameters further and the need for and timing of retreatment, our results suggest that ciliary body PDT may lead to a more selective cyclodestructive technique with potential clinical application in the treatment of glaucoma.

References


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