Verteporfin Photodynamic Therapy in the Rat Model of Choroidal Neovascularization: Angiographic and Histologic Characterization

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PURPOSE. To develop a model of verteporfin photodynamic therapy (PDT) for experimental choroidal neovascularization CNV in the rat.

METHODS. A laser injury model was used to induce experimental CNV in rats. The transit and accumulation of the photosensitizer verteporfin was assessed angiographically in CNV lesions, to determine the optimal time for delivery of light energy. The CNV lesions were then treated with verteporfin PDT, with two doses of verteporfin (3.0 and 6.0 mg/m3) and four activating doses of light energy (10, 25, 50, and 100 J/cm2). Closure of the CNV was assessed both angiographically and histologically. Verteporfin PDT was also performed on areas of normal choroid and retina at the two verteporfin doses and four light energy doses. The effect of these treatments on these structures was also assessed angiographically and histologically.

RESULTS. Peak verteporfin intensities in the CNV were detected at 15 to 20 minutes after intravenous injection. Rates of closure of the CNV varied as a function of the dose of verteporfin and of the activating light energy. Angiographic closure of the CNV correlated with damage to the neovascular complex, as seen with light and electron microscopy. Damage to areas of normal choroid and retina treated with verteporfin PDT also varied as a function of the verteporfin and light energy doses.

CONCLUSIONS. Verteporfin PDT for experimental CNV in the rat is a feasible, effective, and reproducible model that can be used for testing the efficacy of adjunctive therapy to verteporfin PDT. (Invest Ophthalmol Vis Sci. 2002;43:2384–2391)

Photodynamic therapy (PDT) with verteporfin has been shown to be a promising new therapy for the treatment of choroidal neovascularization (CNV) resulting from diseases such as age-related macular degeneration and myopia.1–4 The principle behind this therapy is the use of a photosensitizing agent, such as verteporfin, which preferentially collects in the pathologic neovascular channels.5 Light energy is then used to activate the verteporfin to release oxygen radicals that are toxic to the blood vessels in which the drug has collected. There are, however, limitations to the effectiveness of this therapy. Many patients must undergo more than one treatment to stop the leakage of fluid from the choroidal neovascular membranes, and although the treatment often succeeds in stopping this leakage, patients may still experience a decrease in vision, albeit at a slower rate than without the treatment.5 In addition, previous work on PDT with verteporfin in a nonhuman primate model of CNV has shown treatment-related damage to the surrounding retina, choroid, and retinal pigment epithelium (RPE), and not just destruction of the choroidal neovascular complex.6 The extent of this collateral damage varies as a function of the photosensitizer dose, the magnitude of the activating light energy and the timing of light application. Furthermore, this collateral damage is cumulative with repeated verteporfin PDT treatments.6–8 Antiangiogenic compounds, such as vascular endothelial growth factor, have been shown to be important contributors to the growth of choroidal neovascular membranes.9–12 Antiangiogenic agents such as angiotatin have the ability to inhibit the formation of these neovascular complexes in experimental models of CNV.13 In vitro work has shown that cotreatment with angiotatin when performing PDT using lutetium texaphyrin (Lu-Tex) as the photosensitizing agent causes an increase in the cytotoxic effect of PDT on cultured capillary endothelial cells compared with PDT with Lu-Tex alone.14 This increased cytotoxicity, however, is not seen in cultured RPE cells. This suggests that concomitant administration of antiangiogenic agents may serve as a mechanism of increasing the effectiveness of PDT while minimizing the collateral damage caused by treatment.

There are a myriad of antiangiogenic agents with the potential to serve as adjuncts to PDT. For efficient testing of the efficacy and safety of these various agents, a rapid, reliable, reproducible, and simple animal model of choroidal neovascular membranes and treatment with PDT is essential. In this article, we describe a model of experimental CNV in the rat and the treatment of these neovascular membranes with verteporfin PDT.

METHODS

Induction of Choroidal Neovascular Membranes

All experiments were conducted in accordance with the Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines established by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. Brown-Norway rats were anesthetized with a 0.1 to 0.2 mL of a 50:50 mixture of 100 mg/mL ketamine and 20 mg/mL xylazine. Pupils were dilated with a topical application of 5.0% phenylephrine and 0.8% tropicamide. CNV was experimentally produced with an argon-dye-pulsed laser (model 920; Coherent Medical Laser, Palo Alto, CA) to disrupt Bruch’s membrane using a protocol similar to one previously described.15 The wavelength was 630 nm, and spot size was 100 μm. Power delivered ranged from 130 to 150 mW, applied for 0.1 sec. Typically, six to seven lesions were induced in the left eye of
achieve total energy doses of 10, 25, 50, or 100 J/cm², respectively.

observed.

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The time ranges given in the Results section represent our assessment

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Paramus, NJ) and standard fluorescein filters. One millilitre of 10% fluorescein was injected intraperitoneally, and the timer was started as soon as the fluorescein bolus was injected.

Verteporfin-assisted (QLT Inc., Vancouver, British Columbia, Canada) angiography was performed with the same camera, but with the following verteporfin-specific filters: excitation spectral band centered at 580 nm and fluorescence detection at 695 nm. Maximal gain settings on the digital camera were needed to detect the verteporfin at the study doses. Verteporfin doses were injected into the tail vein. Verteporfin angiography was performed at doses of 1.5, 3.0, and 6.0 mg/m². All doses were delivered in a volume of 0.33 mL, and the appropriate dilutions were made with sterile water. Conversion from weight (in kilograms) to body surface area (in square millimeters) was made, by

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Kramer et al.6 to evaluate the effects of PDT with verteporfin on areas of normal choroid and retina (Table 1). The scale ranged from grade 1 to grade 5. Grade 1 lesions had histologically detectable damage to the RPE, slight damage to the photoreceptor layer with occasional pyknosis in the ONL, and/or closure of the vessels in the choriocapillaris. Grades 2, 3, and 4 lesions showed progressively

Verteporfin PDT in Rat CNV

were evaluated in a masked manner by two experienced graders (ESG, JWM).

Histology

Eyes were enucleated at either 24 hours or 7 days after PDT. Enucleation was performed with animals under deep anesthesia, and then the cornea and lens were removed. The remaining eye cup was placed in a fixative containing 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M cacodylate buffer (pH 7.4) at 4°C overnight. Tissue samples were postfixed in 2% osmium tetroxide, dehydrated in graded ethanol, and embedded in epoxy resin. For light microscopic analysis, 1-μm sections were stained with 0.5% toluidine blue in 0.1% borate buffer and examined with a photomicroscope (Axiophot; Carl Zeiss, Oberkochen, Germany). For electron microscopic analysis, sections were stained with a saturated aqueous uranyl acetate solution and Sato lead stain. Sections were viewed with a transmission electron microscope (model CM 10; Phillips, Eindhoven, The Netherlands).

Figure 1

Late phase of fluorescein angiogram of rat retina 3 weeks after argon-laser induction of CNV. Arrows: areas of fluorescein leakage at the site of the initial laser injury.

Based on the histologic grading scheme described by Kramer et al.6

<table>
<thead>
<tr>
<th>Grade</th>
<th>Damaged Retinal/Choroidal Structures</th>
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<tbody>
<tr>
<td>1</td>
<td>Damage to the RPE, slight damage to the photoreceptor layer, and occasional pyknosis in the ONL, and/or closure of the choriocapillaris</td>
</tr>
<tr>
<td>2</td>
<td>Choriocapillaris closure, damage to the RPE and photoreceptors, and 10%–20% pyknosis in the ONL</td>
</tr>
<tr>
<td>3</td>
<td>Grade 2, with &lt;50% pyknosis in the ONL</td>
</tr>
<tr>
<td>4</td>
<td>Grade 3, with &gt;50% pyknosis in the ONL</td>
</tr>
<tr>
<td>5</td>
<td>Grade 4, with damage to medium and large choroidal vessels, retinal vessels, or inner retinal layers</td>
</tr>
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Photodynamic Therapy

PDT with verteporfin was performed on experimental choroidal neovascular membranes and, in separate animals, on areas of normal choroid and retina. Anesthetized rats were immobilized on a stereotactic frame. PDT was performed 15 to 20 minutes after injection of either 3.0 mg/m² or 6.0 mg/m² verteporfin into the tail vein. The verteporfin was delivered as a bolus in a volume of 0.33 mL. Laser light of 689 nm was administered with a diode laser (Coherent Medical Laser). Laser power at the focal plane was measured with a power meter (Fieldmaster; Coherent, Auburn, CA). The laser spot size was set at 750 μm and was confirmed with a micrometer. The laser spot size on the plane of the retina was the same as that at the plane of the cornea (data not shown). The laser had a constant irradiance of 600 mW/cm², which was delivered for 17, 42, 83, or 166 seconds, to achieve total energy doses of 10, 25, 50, or 100 J/cm², respectively.

Fluorescein angiograms were performed at 24 hours and 7 days after treatment. Some animals were killed at 24 hours for histlogic evaluation. The angiographic data from these animals were included at the 24-hour time point. A choroidal neovascular membrane was defined as being closed after treatment if there was absence of leakage from the treated membrane compared with baseline. All angiograms

each animal. On occasion, the inducing laser burst created an extensive subretinal hemorrhage, and these spots were excluded from any further treatment or analysis. Other lesions in the same eye were included in the study if the subretinal hemorrhage did not extend to within approximately 1 mm of the lesion. The presence of CNV was confirmed at 3 weeks after laser induction by fluorescein angiography. A choroidal neovascular membrane was defined as present if there was early hyperfluorescence with late leakage at the site of the inducing laser injury.

Fluorescin and Verteporfin Angiography

Fluorescin angiography was performed in anesthetized animals with dilated pupils using a digital fundus camera (model TRC 50 IA; Topcon, Paramus, NJ) and standard fluorescein filters. One millilitre of 10% fluorescein was injected intraperitoneally, and the timer was started as soon as the fluorescein bolus was injected.

Verteporfin-assisted (QLT Inc., Vancouver, British Columbia, Canada) angiography was performed with the same camera, but with the following verteporfin-specific filters: excitation spectral band centered at 580 nm and fluorescence detection at 695 nm. Maximal gain settings on the digital camera were needed to detect the verteporfin at the study doses. Verteporfin doses were injected into the tail vein. Verteporfin angiography was performed at doses of 1.5, 3.0, and 6.0 mg/m². All doses were delivered in a volume of 0.33 mL, and the appropriate dilutions were made with sterile water. Conversion from weight (in kilograms) to body surface area (in square millimeters) was made, by using a nomogram developed by Gilpin.16 Peak intensities of verteporfin fluorescence were determined by visual analysis of the angiograms. The time ranges given in the Results section represent our assessment of the time interval over which maximal verteporfin intensities were observed.

Histologic Grading Scheme for PDT’s Effects on Normal Choroid and Retina

We used a histopathologic grading scheme similar to that used by Kramer et al.6 to evaluate the effects of PDT with verteporfin on areas of normal choroid and retina (Table 1). The scale ranged from grade 1 to grade 5. Grade 1 lesions had histologically detectable damage to the RPE, slight damage to the photoreceptor layer with occasional pyknosis in the outer nuclear layer (ONL), and/or closure of the vessels in the choriocapillaris. Grades 2, 3, and 4 lesions showed progressively
greater damage to the choriocapillaris, RPE, and photoreceptors with increasing amounts of pyknotic nuclei in the ONL. Grade 5 lesions showed damage to all the above-mentioned layers and damage to the medium-to-large choroidal vessels or to the retinal vessels or to the cells in the inner retinal layers.

RESULTS

Temporal–Spatial Localization of Verteporfin

Figure 1 shows a typical example of a fluorescein angiogram performed 3 weeks after the laser induction of experimental choroidal neovascular membranes. Late hyperfluorescence was exhibited at the site of the laser injury corresponding to fluorescein leakage from the CNV. All laser-induced breaks in Bruch's membrane resulted in CNV. No spontaneous closure of the experimentally induced CNV was seen during the time course of the experiments. To test the transit of verteporfin through the retinal and choroidal circulations and its collection in the neovascular channels, we performed angiography with three different verteporfin doses: 1.5, 3.0, and 6.0 mg/m². The fluorescence of verteporfin at 1.5 mg/m² was below the resolution of our detection system. With the two higher doses, 3.0 and 6.0 mg/m², we readily detected the flow of dye through the retinal and choroidal circulations. At the earliest time points captured with either of these doses (between 5 and 10 seconds after injection of dye), the verteporfin was already visible in both the choroidal and retinal circulation. Peak intensities of dye in the retinal circulation were visible at approximately 30 to 60 seconds at the 3.0-mg/m² dose (Fig. 2A) and at 15 to 30 seconds at the 6.0-mg/m² dose (Fig. 2B). After these time points, the dye appeared to wash out of the retinal circulation and then wash out of the choroidal circulation. The dye, however, was retained within the experimental neovascular membranes. Peak intensities of dye fluorescence within these neovascular channels were visible between 15 and 20 minutes after induction of both dye doses (Fig. 3A, 3.0 mg/m²; 3B, 6.0 mg/m²).

Verteporfin PDT

Given the maximal pooling of the photosensitizing drug in the choroidal neovascular membranes between 15 to 20 minutes, we decided to administer the activating laser light for PDT during this time interval. Angiographic closure was assessed at 24 hours and 7 days after PDT and was defined as the absence of leakage from the CNV compared with leakage shown in pretreatment fluorescein angiograms (Fig. 4). One feature common to all lesions 24 hours after PDT was the presence of a circle of choroidal hypofluorescence corresponding to the treatment spot size. Lesions that remained open showed early hyperfluorescence in the area of CNV in the center of the treatment area. The hyperfluorescence then spread from the center of the CNV to encompass the whole treatment zone. The intensity of this fluorescence was often similar to that of untreated lesions. In contrast, lesions that responded to PDT did not show any early hyperfluorescence in the CNV (Fig. 4A). Later in the course of the angiogram, there was typically some leakage of fluorescein into the treatment area from the periphery of the zone of hypofluorescence (Fig. 4B).
At day 7 after PDT the spot of choroidal hypo
fluorescence was usually absent (Fig. 5B). Open lesions, however, still displayed early hyperfluorescence followed by late leakage of fluorescein. Closed lesions did not display any fluorescein leakage, but showed some late staining of the treated CNV complex.

Closure rates for PDT at both verteporfin dye doses and all four activating light energy doses are summarized in Tables 2 and 3. At both verteporfin doses there was minimal closure of CNV at 24 hours after PDT when using the 10-J/cm² activating light energy dose, or fluence. The 25-, 50-, and 100-J/cm² doses, however, showed increased closure rates at 24 hours.

The major difference found between the 3.0- and 6.0-mg/m² verteporfin doses was the 7-day posttreatment closure rate. Animals receiving 3.0 mg/m² verteporfin showed no sustained closure of the CNV, except when treated with the highest light energy dose. By contrast, however, the 7-day closure rate for animals injected with 6.0 mg/m² verteporfin increased in a light-dose–dependent manner. The 7-day closure rate in these animals was 6% at the 10-J/cm² light dose and increased to 44% at the 100-J/cm² light dose.

Fluorescein angiography performed 24 hours after PDT in normal choroid and retina showed late fluorescein leakage in the treatment zone (Fig. 6A). This hyperfluorescence was visible after treatment at both doses of verteporfin and at all four light energy doses. The hyperfluorescence was not present on fluorescein angiography performed at 7 days post-PDT (Fig. 6B). The hyperfluorescence was thought to represent fluorescein leakage from the choroidal vessels through the damaged RPE layer, as previously described in primate models of verteporfin PDT.

**Histology of Untreated CNV**

Figure 7A shows a light microscopic picture of untreated CNV three weeks after induction with the argon dye laser. There was a gross destruction of the outer retinal layers, RPE, and Bruch’s membrane, secondary to the laser energy used to induce the CNV. Macrophages were seen in this zone of disrupted retina. The CNV complex contained a mixture of fibrous elements and open vascular channels. These vascular channels appeared to emerge from feeder vessels that protruded from the choroid through the disrupted area of Bruch’s membrane into the subretinal space. Electron microscopy confirmed the presence of the open vascular channels in the choroidal neovascular complex with intact endothelial cells.
TABLE 2. Closure Rate of Experimental Choroidal Neovascular Membranes 24 Hours and 7 Days after Treatment with 3.0 mg/m² Verteporfin and Various Light Energy Doses

<table>
<thead>
<tr>
<th>Light Fluence (J/cm²)</th>
<th>Lesions Closed at 24 Hours</th>
<th>Lesions Closed at 7 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>4/14 (29)</td>
<td>0/11 (0)</td>
</tr>
<tr>
<td>25</td>
<td>12/13 (92)</td>
<td>0/8 (0)</td>
</tr>
<tr>
<td>50</td>
<td>15/21 (71)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>100</td>
<td>4/7 (57)</td>
<td>3/6 (50)</td>
</tr>
</tbody>
</table>

Data show number of lesions closed of the total examined, with percentage closed in parentheses.

Histology of Treated CNV

Figure 8A shows a section of a choroidal neovascular membrane treated with 6.0 mg/m² verteporfin and fluence of 25 J/cm² at 24 hours after PDT. This lesion was defined as closed, based on fluorescein angiographic analysis. All lesions defined as closed, regardless of verteporfin or light energy dose, shared similar histologic features. One prominent feature of closed lesions was the vacuolization of the endothelial cells. There was collapse and closure of the vascular channels in the CNV complex, as well as in the underlying choroid. Fibrin was found in extravascular spaces, further supporting the finding of damage to the vessel walls. In some sections macrophages were seen migrating into the treated choroidal neovascular complex. Electron microscopy (Figs. 8B, 8C) confirmed the vacuolization and collapse of the endothelial cells, release of fibrin into the extravascular space, and thrombus formation within the lumen of the vessels.

Histologic analysis of an angiographically closed lesion 7 days after PDT shows the organization of the CNV complex into a scar (Fig. 8D). Fibrous tissue proliferated within the CNV complex and there was an identifiable plane separating the retina from the underlying lesion. An occasional patent vascular channel was visible within the scar complex.

Histology of Treated Choroid and Retina

Verteporfin PDT to areas of normal choroid, retina, and RPE resulted in damage to these structures at all drug and light doses tested. According to the histopathologic grading scheme outlined in Table 1, a pattern of greater damage to these structures was seen with increasing verteporfin and light dose levels. Figure 9A shows a section of an eye treated with 3.0 mg/m² verteporfin and fluence of 10 J/cm². There was some damage to the RPE layer, mild disruption of the photoreceptor cell layer, and ONL pyknosis in approximately 10% to 20% of the cells. The damage in this lesion was classified as grade 2. Doubling the verteporfin dose to 6.0 mg/m² but maintaining the fluence at 10 J/cm² showed a greater extent of damage (Fig. 9B). There was closure of vessels in the choriocapillaris, greater disruption of the RPE, and marked photoreceptor outer and inner segment disruption and edema, and more than 50% of the ONL showed pyknosis. The inner retinal layers and the larger choroidal vessels, however, showed no damage, and the damage in this lesion was classified as grade 4. Finally, increasing the light energy to 100 J/cm² with the 6.0 mg/m² dose of verteporfin caused the greatest amount of damage (Fig. 9C). In addition to the damage described in the previous lesion, there was also closure of retinal vessels and medium-sized choroidal vessels and pyknotic nuclei present in the inner retinal layers. The extensive destruction put this lesion into grade 5.

DISCUSSION

PDT with verteporfin has become a widely accepted and frequently performed therapy in patients with CNV of a variety of causes. To date, however, all in vivo work with verteporfin PDT for CNV has been conducted in nonhuman primates or in human clinical trials. Dobi et al. first proposed the rat model for laser-induced experimental CNV in 1989. Various aspects of CNV have been studied with this model, and this technique has also been transferred to the mouse. To the best of our knowledge, however, this is the first application of verteporfin PDT in the rat model.

Our goal was to create a simple, practical, and reproducible method for performing verteporfin PDT in a rodent system for the purpose of testing adjunctive and combination therapies that might increase the efficacy of verteporfin PDT. In addition to ease of use and reproducibility, this model also had to correlate well with the known features of verteporfin PDT in primates and humans. The model presented herein meets these criteria.

The first major analogy between our model and the primate models and human disease is the temporal-spatial localization of the verteporfin dye within the choroidal neovascular membranes. Work in cynomolgus monkeys has shown that the optimal time for light energy delivery is approximately 20 minutes after the injection of verteporfin. Activation at this time after injection, regardless of the verteporfin dose used, resulted in the highest CNV closure rates and the least amount of collateral damage to surrounding normal retina, choroid, and RPE. This time for light energy delivery has since translated into clinical application in humans. In current clinical practice, the laser energy is delivered 15 minutes after drug infusion is begun. In the model presented herein, angiography showed the peak concentration of the photosensitizer in the CNV also to occur at approximately 15 to 20 minutes. This suggests similar dynamics of drug flow through the ocular vasculature in our model compared with the human condition. Future experiments with adjuvants that could affect this dynamic would thus be possible in this model.

Our rat model has a second feature in common with the previously developed monkey models and the human clinical situation. The data presented herein establish the presence of a measurable dose–response curve for the closure of CNV as a function of either verteporfin or light doses at 7 days after treatment. Experiments in monkeys and human clinical trials have shown an optimal drug dose of 6.0 mg/m², irradiance of 600 mW/cm², and fluence of 50 J/cm² delivered over 83 seconds. These parameters provide the maximal therapeutic effect of PDT while minimizing the collateral damage to surrounding normal structures. It is unclear why this correlation is less preserved at the 24-hour time point.

Angiographic closure of the CNV lesion in the rat model also shows a drug and light dose dependency, especially at the 1-week time point. Light doses of 10 J/cm², at either drug dose did not effect a significant closure rate. Increasing the light energy level to between 25 and 100 J/cm² increased the closure rates. The differences in the closure rates between 3.0 and

TABLE 3. Closure Rate of Experimental Choroidal Neovascular Membranes 24 Hours and 7 Days after Treatment with 6.0 mg/m² Verteporfin and Various Light Energy Doses

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<tr>
<td>25</td>
<td>10/12 (83)</td>
<td>3/9 (33)</td>
</tr>
<tr>
<td>50</td>
<td>6/11 (54)</td>
<td>4/11 (36)</td>
</tr>
<tr>
<td>100</td>
<td>5/12 (42)</td>
<td>4/9 (44)</td>
</tr>
</tbody>
</table>

Data are described in Table 2.
6.0 mg/m² were most pronounced at the 7-day period, with the 6.0 mg/m² dose having more of an effect at this time point at lower doses of light energy. Furthermore, we found greater persistent closure of the experimental CNV lesion at 7 days after treatment with the higher verteporfin dose of 6.0 mg/m² than with the dose of 3.0 mg/m². The presence of a detectable and dose–response curve is crucial to the testing of adjuvant therapies, because we now can monitor for shifts in these

**FIGURE 6.** (A) Late phase of fluorescein angiogram taken 24 hours after PDT treatment of normal choroid and retina with 6.0 mg/m² verteporfin. The superior area was treated with fluence of 50 J/cm² and the inferior area with 100 J/cm². (B) Late phase of fluorescein angiogram in same animal as shown in (A) taken 7 days after treatment.

**FIGURE 7.** (A) Light micrograph of untreated CNV showing a projection of a vascular frond through the level of Bruch’s membrane (arrow). The CNV contained many capillaries (arrowhead) and was situated below a disrupted outer retina containing a cluster of macrophages (*). Toluidine blue; magnification, ×40. (B) Electron micrograph of the edge of an untreated CNV lesion enveloped by proliferating RPE (P). A patent capillary is shown with a prominent endothelial cell (E), along with microvilli (✱) prominent on native (R) and proliferating (P) RPE. Arrow: Bruch’s membrane. os, outer segments. (C) Electron micrograph of a capillary in an untreated CNV lesion, showing prominent endothelial cells (E), proliferating RPE cells (P) with microvilli (✱), active fibroblasts (F), and collagen (Co). Bar: (B) 5 µm; (C) 2 µm.
response curves as a function of different pharmacologic agents. Successful adjuvants to PDT would cause an increase in the CNV closure rate at the lower drug dose and lower light energy doses.

Histologic analysis of untreated and treated lesions extends the analogous nature of the rat model to the monkey model and to human disease. Laser-induced CNV in the rats showed morphologic features common to other models of CNV. There were vascular channels within a complex of fibrous proliferation, extending inwardly from the choroid through a gap in Bruch’s membrane. Verteporfin PDT caused destruction of the vascular channels and organization of the CNV complex into a scar. These histopathologic features correlated well with angiographic closure of the CNV. There does not, however, have to be complete destruction of all the vessels in the CNV to effect an angiographically definable closure. The organization of the CNV complex into a scar, as was seen in the sections taken 7 days after treatment, appeared to create an effect barrier to the leakage of fluorescein from the remaining abnormal vascular elements. The laser used to induce the CNV created a disruption of the Bruch’s membrane and RPE, two structures that are important in the functional separation of the choroid from the retina. The post-PDT scar complex appeared, at least on histologic examination, to restore some of the pre-CNV induction separation between the choroid and the retina. This may be why not all the vessels of the CNV complex have to be closed by the PDT treatment to show angiographic closure.

Collateral damage to the normal retina, choroid, and RPE also showed a drug and light dose dependency in our model, in a consistent and measurable manner. One limitation of the current PDT treatment regimen was the damage to normal structures, thus potentially limiting the number of repeat treatments and drug and light doses that could be applied safely. Our model would allow for the reproducible and rapid histologic assessment of adjuvant therapy on the collateral damage caused by verteporfin PDT.

In summary, the data presented here support the use of experimental CNV treatment with verteporfin PDT in the rat as a valid and effective model of verteporfin PDT in humans. This model can serve as a reasonable first step in rapid analysis of potential therapies for CNV treatment and for assessing the effects of such therapies on both normal and abnormal structures, before proceeding to primate studies or to human clinical trials.
the RPE and choriocapillaris at the level of Bruch's membrane (arrow) were visible in the ONL (O), although the outer and inner segments (●) appeared intact. Damage was noted in the RPE and choriocapillaris around Bruch's membrane (arrow). I, inner nuclear layer (INL). (B) Light micrograph of an area of normal choroid and retina treated 24 hours after PDT with 6.0 mg/m² verteporfin and fluorescence of 10 J/cm². The lesion was classified as grade 4. Most of the ONL (O) in the area of treatment was pyknotic, and the outer and inner segments (●) were severely damaged. RPE and choriocapillaris at the level of Bruch’s membrane (arrow) were damaged. The INL (I) appeared unchanged. (C) Light micrograph of an area of normal choroid and retina treated 24 hours after PDT with 6.0 mg/m² verteporfin and fluorescence of 10 J/cm². The lesion was classified as grade 5. The entire ONL (O) was damaged, as were the outer and inner segments (●) and the RPE and choriocapillaris at the level of Bruch’s membrane (arrow). Choroidal vessels (open arrow) and retinal vessels (arrowheads) were also damaged, and pyknosis (small filled arrows) was visible in the INL (I). (A, B, C) Toluidine blue; magnification, ×40.

References


