Effects of Photodynamic Therapy Using Verteporfin on Experimental Choroidal Neovascularization and Normal Retina and Choroid up to 7 Weeks after Treatment

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PURPOSE. To study the long-term effects of photodynamic therapy (PDT), using liposomal benzo-porphyrin derivative (BPD) or Verteporfin, on experimental choroidal neovascularization (CNV) and on normal retina and choroid (with no CNV) in the cynomolgus monkey eye.

METHODS. Photodynamic therapy was performed in 8 cynomolgus monkey eyes with experimental CNV induced by laser injury. The effect of PDT on normal retina and choroid (with no CNV) was studied in 9 monkey eyes. Liposomal BPD was administered intravenously (0.375 mg/kg) either as a bolus, as a slow infusion over 32 minutes, or as a fast infusion over 10 minutes. Photodynamic therapy was performed using light at a wavelength of 689 or 692 nm, with an irradiance of 600 mW/cm^2 and fluence of 150 J/cm^2. Follow-up studies, including fundus photography and FA, were performed at 24 hours after PDT and then weekly. Indocyanine green and BPD angiography were performed in selected cases. Tissues were examined with light and electron microscopy at the end of follow-up.

RESULTS. Twenty-three of the 32 areas of CNV treated with PDT showed absence of angiographic leakage at 24 hours. Twenty-eight areas of CNV were followed for 4 weeks; 22 of 28 showed absence of angiographic leakage at 2 weeks; and 20 of 28 at 4 weeks of follow-up. Forty spots on the normal retina and choroid were treated with PDT and were followed for 4 to 7 weeks. These spots showed pigment-laden cells in the outer retina, variably pigmented retinal pigment epithelium (RPE) in the treated area, intact neurosensory retina, and reperfusion of the choriocapillaris.

CONCLUSIONS. Photodynamic therapy leads to absence of angiographic leakage for at least 4 weeks in experimental CNV in the monkey model. In the normal monkey eye the RPE and choriocapillaris show generalized recovery with preservation of the neurosensory retina 7 weeks after PDT. (*Invest Ophthalmol Vis Sci.* 1999;40:2322–2331)

Photodynamic therapy (PDT) is a developing treatment modality. It involves intravenous injection of a photosensitizer that accumulates in the neovascular and tumor tissue. This photosensitized tissue is then irradiated by light at the absorption maximum of the dye leading to cytotoxicity.[1,2]

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Previous work in our laboratory has shown effective treatment at 24 hours after PDT, of experimental choroidal neovascularization (CNV), using a liposomal preparation of benzoporphyrin derivative (BPD) or Verteporfin.[3–5] Optimal treatment parameters were identified that led to an absence of angiographic leakage and histologic occlusion of CNV. In normal areas of the fundus treated at these parameters, PDT caused choriocapillaris closure with minimal damage to the outer retina. This included disruption of the retinal pigment epithelium with minimal pyknosis of the outer nuclear layer and swelling of the outer segments.[4–6] Verteporfin is a safe drug for human use and has been used in clinical trials in dermatology.[7] Therefore, this treatment modality offers the potential of selective closure of CNV with minimal damage to the overlying retina.

Age-related macular degeneration (AMD) is the leading cause of blindness in the elderly in the western world.[8] Choroidal neovascularization causes 90% of the visual loss in AMD.[9] Currently, the only established management of subfoveal CNV is laser photocoagulation, with inherent drawbacks including the risk of a sudden decrease of vision, and significant recurrence of CNV after the treatment. Additionally, only 13% cases of neovascular AMD are eligible for laser treatment under the present guidelines.[10] Therefore, there is a need for alternative treatment modalities for this condition. Other treatments under investigation include low-dose external beam and proton

TABLE 1. PDT Treatment Parameters

Area Treated	No. of Eyes	No. of Areas	Mode of Dye Administration	PDT Spot Size, μm	Duration of Follow-up
CNV	4	20	Bolus	1250	4 wk
CNV	3	11	Slow infusion	1250	4 wk (2 eyes) 1 wk (1 eye)
CNV	1	1	Fast infusion	4000	5 wk
Normal R & C	2	8	Bolus	1250	7 wk & 5 d
Normal R & C	5	28	Slow infusion	1250 1875 (1 eye)	3 wk (3 eyes) 4 wk (2 eyes)
Normal R & C	2	4	Fast infusion	4000	5 wk (1 eye) 2 wk (1 eye)

R & C, retina and choroid.

irradiation, systemic thalidomide and other antiangiogenic agents, and PDT.

Before starting the clinical trials of photodynamic therapy for AMD, we investigated the "long-term" (4 to 7 weeks) effect of the treatment on tissues surrounding the CNV, namely the neurosensory retina and choroid, and the persistence of CNV closure. We performed the studies with Verteporfin in cynomolgus monkeys, in eyes with experimental CNV, and on normal eyes without CNV.

MATERIALS AND METHODS

Animals

Twelve Maccaca Fascicularis cynomolgus monkeys were used in accordance with the ARVO Statement on the Use of Animals in Vision and Ophthalmic Research, and in compliance with the guidelines developed by the Animal Care Committee of the Massachusetts Eye and Ear Infirmary. Eight eyes of 7 monkeys were used to study the effect on CNV, and 9 eyes of 5 monkeys were used for the study of normal retina and choroid. Monkeys (3 to 5 kg) were anesthetized for all procedures using ketamine hydrochloride (20 mg/kg), acepromazine maleate (0.25 mg/kg), and atropine sulfate (0.125 mg/kg), as previously described.[3–5] At the end of the experiment enucleation was performed under deep anesthesia, and animals were euthanatized using pentobarbital (50 mg/kg), as previously described.[4,5]

Induction of CNV

Choroidal neovascularization was created in 8 monkey maculae, as previously described, by inducing damage to the outer retina using high-intensity argon laser.[3–5,11,12] The monkeys were followed weekly with fundus photography and FA to detect the development of CNV.

Liposomal BPD Verteporfin Administration

Liposomal BPD or Verteporfin was provided by QLT Phototherapeutics (Vancouver, British Columbia, Canada). The Verteporfin was handled, reconstituted and stored according to the guidelines provided by the manufacturer, and protected from light at all times as previously described.[4,5] The Verteporfin was infused as an intravenous bolus (0.375 mg/kg or approximately 6 mg/m^2) over 30 seconds followed by a flush[4] in 3 animals for PDT of CNV and in 1 animal for PDT of normal

retina and choroid. The Verteporfin was infused (0.375 mg/kg) over 31.9 minutes (slow infusion) via an infusion pump (IVAC 70 syringe pump; IVAC, San Diego, CA),[5] in 3 animals for PDT of CNV and in 3 animals for PDT of normal retina and choroid. One of the animals in the slow infusion group received a Verteporfin dose of 0.75 mg/kg. The Verteporfin was infused intravenously over 10 minutes (fast infusion)[5] in 1 animal for PDT of CNV and in 1 animal for PDT of normal retina and choroid. The infusion rates were selected from ongoing trials of PDT using Verteporfin for dermatology trials and from trials proposed for AMD.

Lasers

Laser irradiation was performed in initial experiments with laser light at 692 nm using Argon/Dye laser (Coherent 920; Coherent Medical Laser, Palo Alto, CA) and subsequently at 689 nm using an Ocular Photoactivation Diode Laser (Coherent Medical Laser). The laser light was delivered via slit lamp using an adaptation of the ophthalmic delivery systems called the Laser Link Photoactivation Slit Lamp delivery system (patent No. 5336216, Coherent Medical Laser).

Photodynamic Therapy

The light parameters were selected on the basis of previous investigations[4,5] and were kept constant for all experiments at an irradiance of 600 mW/cm^2 and fluence of 150 J/cm^2. Light was focused on the retina using a plano contact lens (OGFA; Ocular Instruments, Bellevue, WA) producing either a 1250-, 1875-, or 4000-μm spot, which was centered on the area to be treated. Areas of CNV were identified using a fluorescein angiogram done within 48 hours before the planned PDT. Areas of normal retina and choroid were selected in the posterior pole, in areas that could facilitate identification during histologic preparation. Irradiation was performed 10 to 71 minutes after the start of the intravenous dye injection. The follow-up at 24 hours and at 2 and 4 weeks was done by fundus photography and FA, and the findings were confirmed at 4 to 7 weeks by histopathology.

Eight monkey eyes with experimental CNV were treated with PDT (Table 1). Photodynamic therapy was performed in 4 eyes with CNV using a bolus administration of Verteporfin and followed for 4 weeks. Photodynamic therapy was done in 3 eyes with CNV, using a slow infusion of Verteporfin and followed for 1 to 4 weeks. Photodynamic therapy was done in 1 eye with CNV, using a fast infusion of dye and followed for
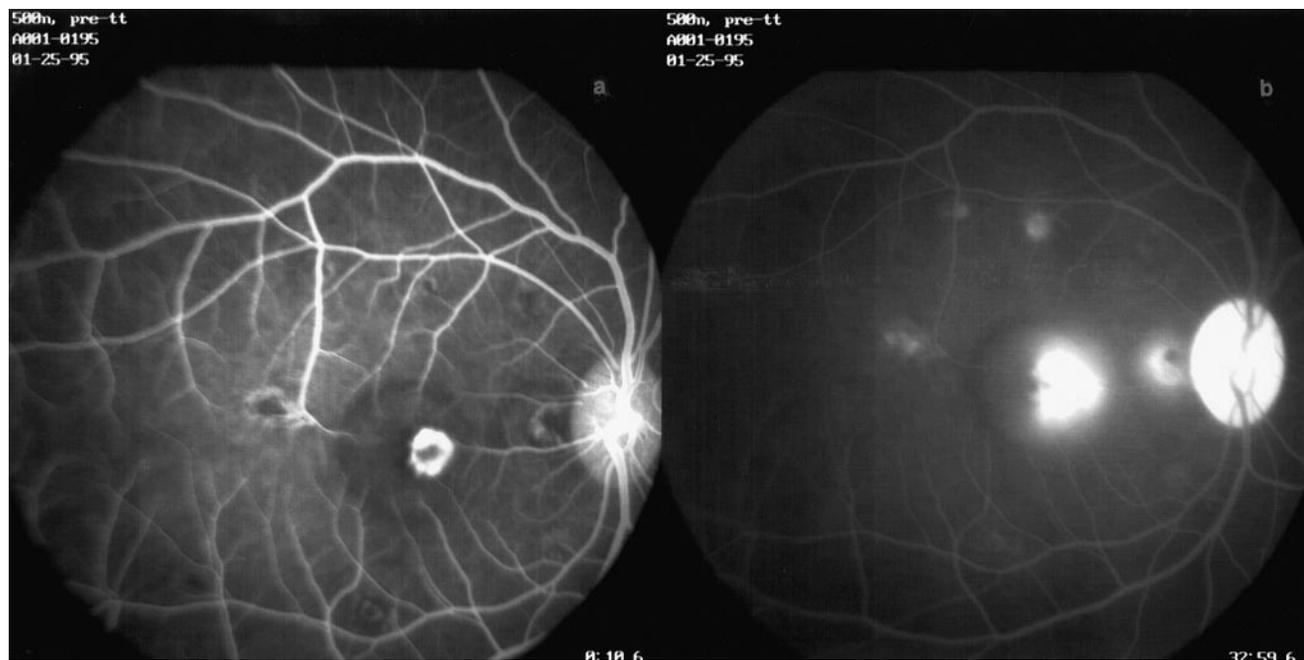
FIGURE 1. (**A**) Early-phase fluorescein angiogram of the CNV model showing hyperfluorescence in the area of experimental CNV. (**B**) Late-phase fluorescein angiogram of the CNV model showing leakage in the area of experimental CNV.

5 weeks. The effect of PDT on the normal retina and choroid was studied by performing PDT on 9 normal monkey eyes (Table 1). Photodynamic therapy was done in 2 eyes of 1 animal after bolus injection of Verteporfin and was followed for 7 weeks and 5 days after PDT. Photodynamic therapy using a slow injection of Verteporfin was done in 5 eyes of 3 animals, with follow-up for 3 or 4 weeks. Photodynamic therapy was performed in 2 eyes of 1 animal using a fast (10-minute) infusion of Verteporfin and a larger treatment spot size of 4000 μm, with follow-up for 2 or 5 weeks.

Photography

Findings were documented by color fundus photography and fluorescein angiography (FA) performed at 24 hours after PDT,
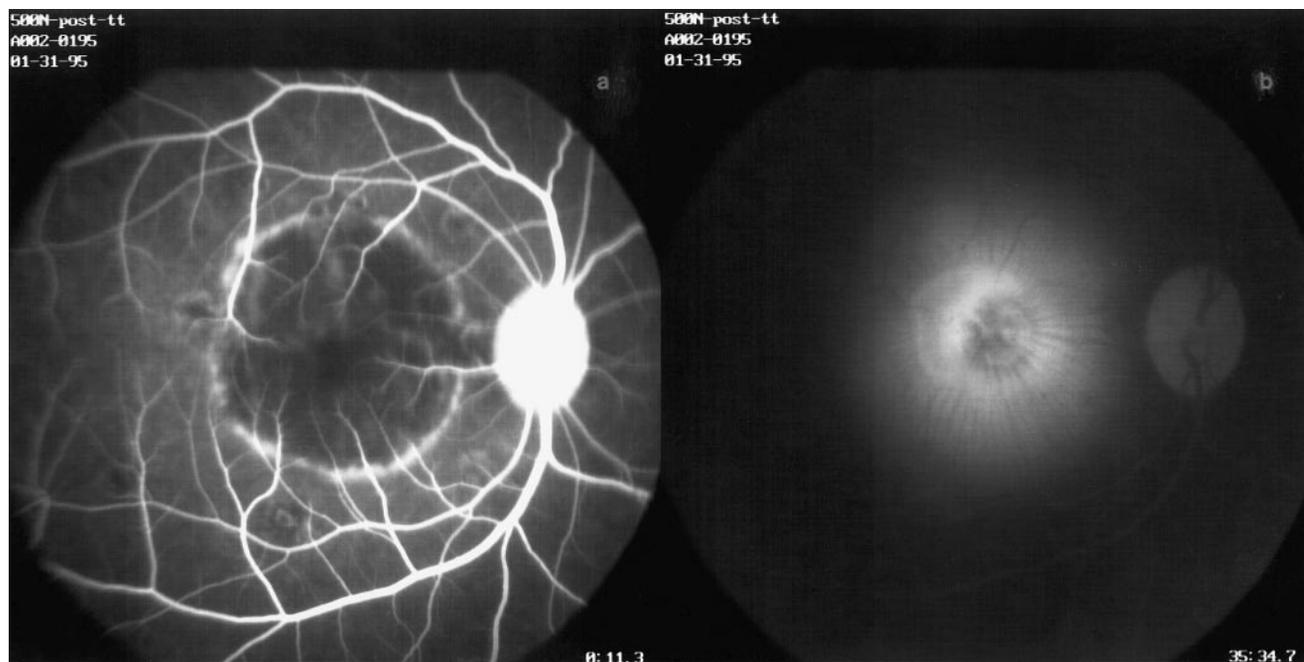

FIGURE 2. (**A**) Early-phase fluorescein angiogram taken 24 hours after PDT of experimental CNV, showing hypofluorescence of the treatment spot. Normal retinal circulation is seen overlying the hypofluorescence. (**B**) Late-phase fluorescein angiogram taken 24 hours after experimental PDT of CNV, showing hyperfluorescence of the PDT-treated area.
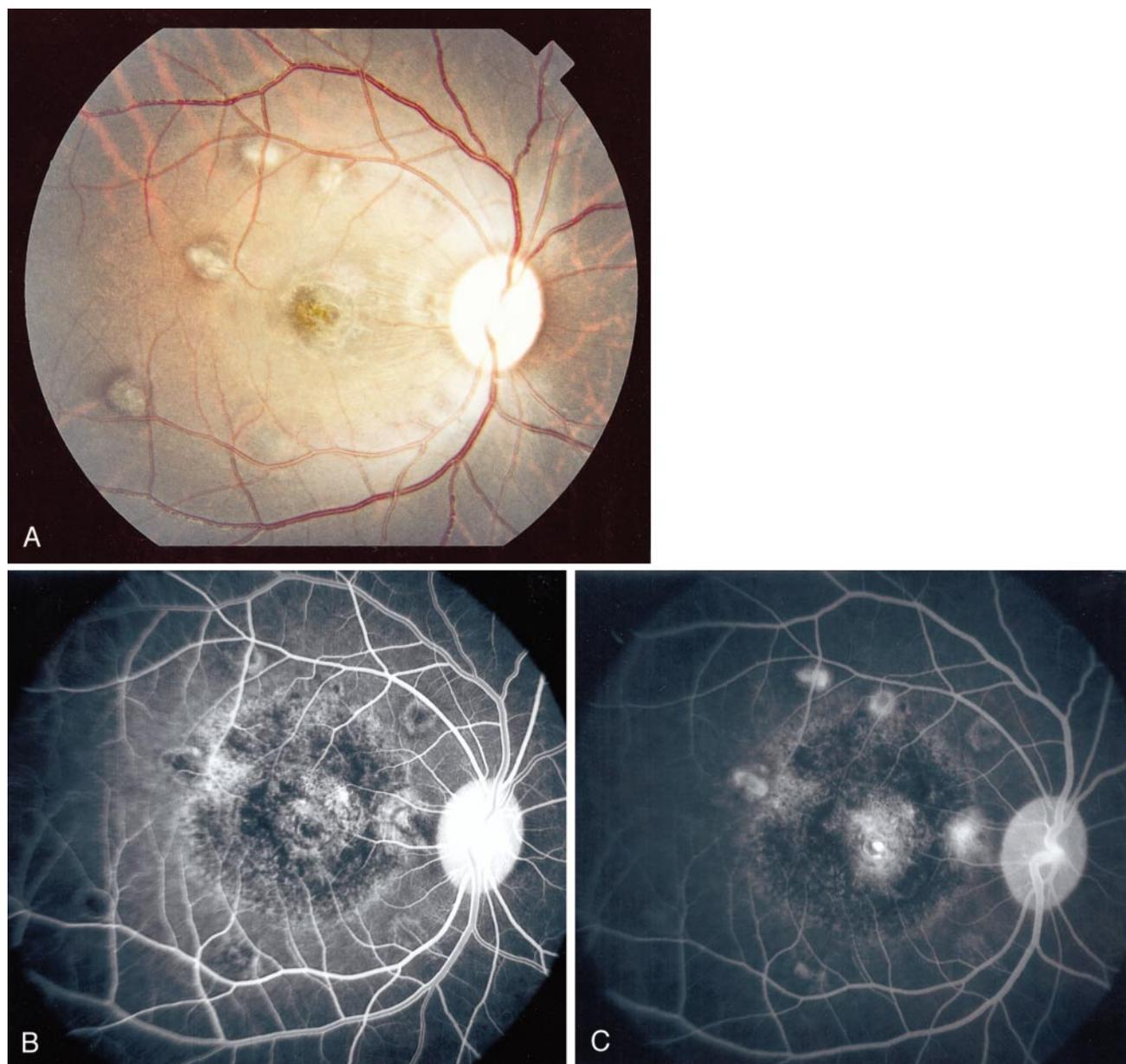
FIGURE 3. (**A**) Color fundus photograph of the monkey eye taken 5 weeks after PDT of CNV, showing minimal hypopigmentation in the treated area and some hyperpigmentation of the treated CNV. (**B**) Early-phase fluorescein angiogram taken 5 weeks after PDT of CNV, showing mottled hypo- and hyperfluorescence of the PDT-treated area. (**C**) Late-phase fluorescein angiogram taken 5 weeks after PDT of CNV, showing mottled hyperfluorescence of the treated CNV with persistent hypofluorescence of the PDT-treated area.

and every week in follow-up. The 50 IA fundus camera (Topcon, Paramus, NJ) and Imagenet system (Topcon) were used to digitally capture the image, or a CF-60 Z camera (Canon, Lake Success, Long Island, NY) were used for angiography on film. Fluorescein angiography was performed in all cases with an intravenous injection of 0.1 ml/kg body weight of 10% sodium

TABLE 2. Efficacy of PDT: 2- and 4-Week Follow-up

Time of Irradiation*	Follow-up at 24 Hours after FA Closure	Follow-up at 2 Weeks after FA Closure	Follow-up at 4 Weeks after FA Closure
<20	6/7	5/6	5/6
20–50	13/16	12/14	10/14
>50	4/9	5/8	5/8
Total	23/32	22/28	20/28

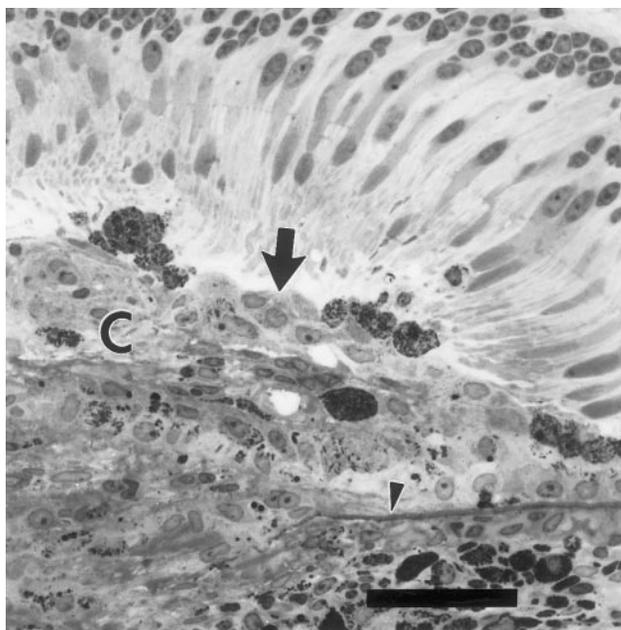* Minutes after start of dye injection.

FIGURE 4. Light micrograph of CNV at 4 weeks after PDT. CNV is covered by surviving outer nuclear layer, inner segments, and a few rudimentary outer segments. There is a layer of proliferated RPE (*arrow*) enveloping the CNV. Pigment-laden cells are seen overlying the proliferated RPE. The CNV (C) extends through Bruch's membrane (*arrowhead*) and has a few capillaries. The spaces seen are acinar structures in RPE cells.[11] Scale bar, 50 μm.

fluorescein, and images were obtained for up to 30 minutes after dye injection. Angiography was performed with indocyanine green (ICG) and liposomal BPD or Verteporfin, in 5 animals before and after PDT of CNV, and in 1 animal with PDT of the normal retina and choroid. For Verteporfin angiography, Verteporfin was injected at a dose of 2 mg/kg using a bolus intravenous injection over 30 seconds. For ICG angiography, ICG was injected at a dose of 0.75 mg/kg via the saphenous vein. Angiographic images were obtained for 60 minutes after injection of the dye. The dye was injected, and photographs were obtained for ICG and Verteporfin every 2 to 3 seconds for the first 30 seconds, then at 45 seconds, and at 1, 2, 3, 5, 7, 10, 15, 30, 45, and 60 minutes using the Topcon 50 IA fundus camera, BPD (Verteporfin) filters, and Topcon Imagenet System. The fluorescein angiograms were graded by three observers (DH, ESG, JWM) to determine the effects of treatment. Effective treatment of CNV was characterized by loss of angiographic leakage from CNV on FA. In these cases the CNV did not perfuse or leak on angiography, although lesions showed hyperfluorescence in the late frames of the angiogram beginning at the periphery of the PDT treatment spot.

Histology

All eyes were enucleated at the end of the experiment. The enucleated eyes were bisected and placed in modified Karnovsky's fixative at 4°C overnight then transferred to 0.1 M cacodylate buffer at pH 7.4. Tissues were postfixed in buffered 2% osmium tetroxide, dehydrated in graded ethanols, and embedded in Epon. One-micrometer-thick sections were stained with 0.5% toluidine blue in borate buffer for light microscopy and examined using a Zeiss photomicroscope (Axiophot,

Oberkochen, Germany). Thin sections were stained with uranyl acetate in methanol, and Sato's lead stain, and examined with a Phillips CM 10 transmission electron microscope (Eindhoven, The Netherlands).

RESULTS

Efficacy of PDT

The efficacy of CNV closure was demonstrated by the absence of angiographic leakage with FA. Typically the CNV did not perfuse, and there was hypofluorescence in the early phase of the angiogram, with no leakage from CNV as the angiogram progressed. Hyperfluorescence was noted in the PDT treatment spot starting from the periphery of the treated area in the late phase of the angiogram. Figure 1 demonstrates CNV before PDT, which shows hyperfluorescence in the early phase of the angiogram, followed by leakage of the dye in the late phase. Figure 2 demonstrates an absence of angiographic leakage from the CNV, 24 hours after PDT.

Photodynamic therapy of CNV performed after intravenous injection of liposomal BPD (Verteporfin) either as a bolus, as a slow infusion over 31.9 minutes, or as a fast infusion over 10 minutes, led to the following observations (as shown in Table 2).

At 24 hours after PDT of CNV ($n = 32$) the following observations were made on FA. Choroidal neovascularization irradiated within 20 minutes of the beginning of Verteporfin injection showed absence of angiographic leakage in 6 of the 7 CNV treated. Choroidal neovascularization irradiated 20 to 50 minutes after the beginning of Verteporfin injection showed an absence of angiographic leakage in 13 of the 16 CNV that were treated. Choroidal neovascularization irradiated after 50 minutes of the beginning of Verteporfin injection showed lack of angiographic leakage in 4 of the 9 treated CNV.

Follow-up at 2 weeks after PDT of CNV ($n = 28$) demonstrated the following observations by angiography. Choroidal neovascularization irradiated within 20 minutes of the beginning of dye infusion continued to show an absence of angiographic leakage in 5 of the 6 CNV that were treated and followed. Choroidal neovascularization irradiated 20 to 50 minutes after the beginning of Verteporfin injection continued to show absence of angiographic leakage in 12 of the 14 CNV that were treated and followed. However, CNV irradiated more than 50 minutes after the beginning of Verteporfin injection showed a lack of angiographic leakage in 5 of the 8 CNV treated and followed.

Follow-up at 4 weeks after PDT of CNV ($n = 28$) demonstrated the following observations by angiography. Choroidal neovascularization irradiated within 20 minutes of the beginning of Verteporfin injection showed an absence of angiographic leakage in 5 of the 6 treated CNV. Choroidal neovascularization irradiated 20 to 50 minutes after the beginning of Verteporfin injection showed an absence of angiographic leakage in 10 of the 14 CNV that were treated and followed. Choroidal neovascularization irradiated more than 50 minutes after the beginning of Verteporfin injection showed a lack of angiographic leakage in only 5 of the 8 treated CNV.

Light (Fig. 4) and electron microscopy of the experimental CNV that showed an absence of angiographic leakage at 4 weeks of follow-up demonstrated envelopment of the CNV by proliferating hypopigmented retinal pigment
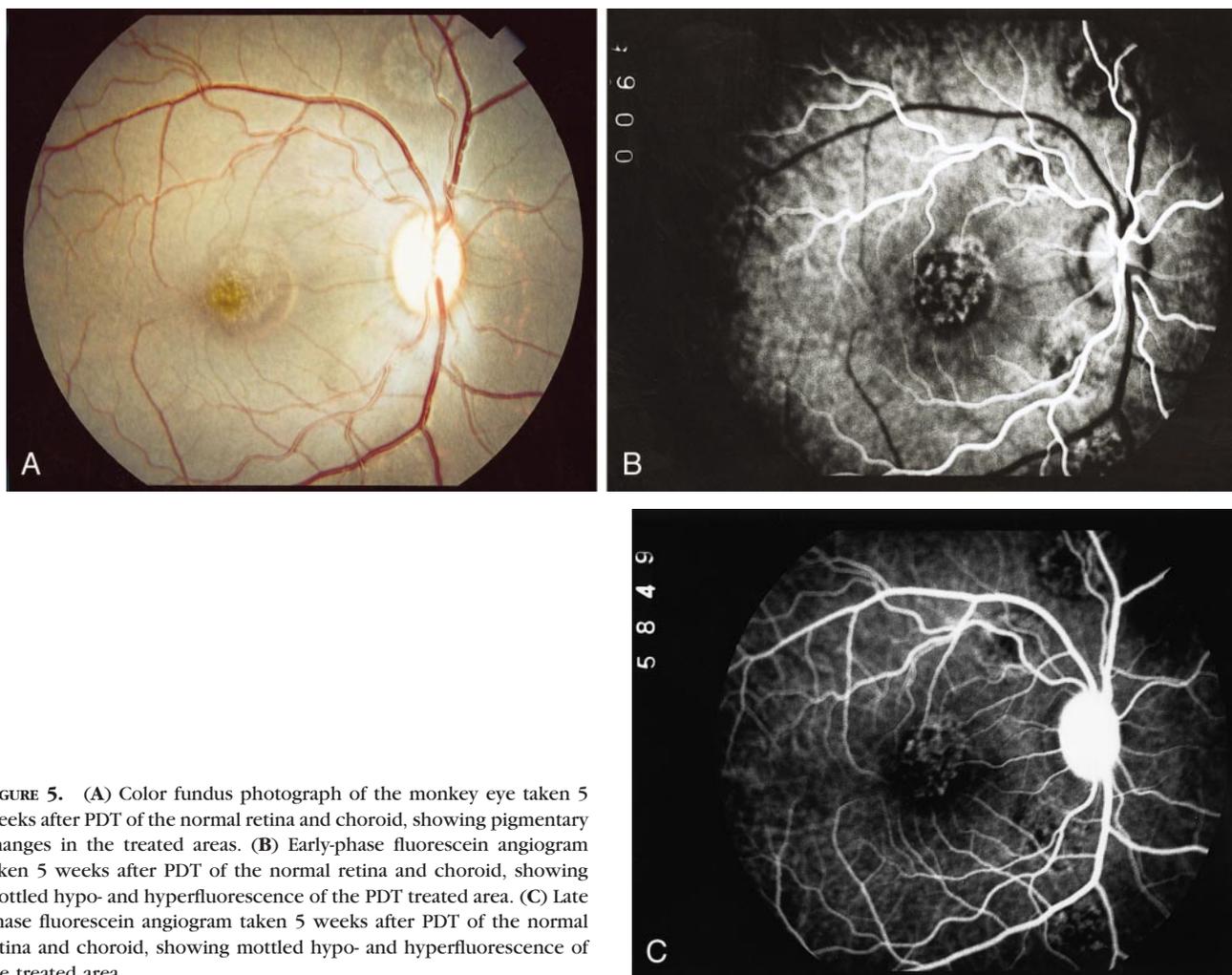
FIGURE 5. (**A**) Color fundus photograph of the monkey eye taken 5 weeks after PDT of the normal retina and choroid, showing pigmentary changes in the treated areas. (**B**) Early-phase fluorescein angiogram taken 5 weeks after PDT of the normal retina and choroid, showing mottled hypo- and hyperfluorescence of the PDT treated area. (**C**) Late phase fluorescein angiogram taken 5 weeks after PDT of the normal retina and choroid, showing mottled hypo- and hyperfluorescence of the treated area.

epithelium (RPE). These proliferating RPE cells were seen connecting to the native RPE at the margin of the treated CNV. Rare open capillaries were seen. There were pigment-laden cells in the subretinal space as well as within the CNV. The treated experimental CNV also contained RPE cells and fibroblasts. Acinar structures were seen in the RPE cells, as previously described.[13] The overlying neurosensory retina and retinal vessels appeared mostly undamaged, although the laser injury used to create the experimental CNV caused some disruption of outer and inner nuclear layers, similar to that in lesions examined 24 hours after PDT as previously reported.[4,5]

Selectivity of PDT

The selectivity of PDT was studied by performing PDT on the normal monkey retina and choroid in 9 eyes of 5 monkeys. The follow-up was done by fundus photography and FA, and findings were confirmed at the end of follow-up by light and electron microscopy. The findings observed were similar for all Verteporfin doses and treatment spot sizes. Fundus photographs at 24 hours after PDT showed mild graying of the treatment area, and FA showed early hypofluorescence of the spot with increasing hyperfluorescence in the mid and late phases of the angiogram. One week after PDT there was a minimal pigmentary change on fundus photographs, and FA

demonstrated early hypofluorescence with late mottled hypo- and hyperfluorescence. At 2, 3, and 4 weeks after PDT, color fundus photographs showed minimal pigment mottling in the treated area (Fig. 5A), and FA showed mottled hypo- and hyperfluorescence both in the early (Fig. 5B) and late (Fig. 5C) phases of the angiogram. At 7 weeks after PDT, fundus photography did not show any visible change in the treatment areas, and FA showed no change in the early phase of the angiogram, but in the late-phase minimal mottled hypo- and hyperfluorescence were seen.

Light and transmission electron (Fig. 6) microscopic examination of the treated areas at 4 weeks of follow-up showed occasional pyknosis in the inner retina and mild disarray of outer segments with the rest of the retina appearing normal. Retinal vessels looked normal. The RPE was single layered with variable pigmentation. Pigment-laden cells with evidence of phagocytic activity were seen overlying the RPE in the irradiated area. RPE cells were polarized with apical villi containing pigment granules and disorganized basal folds. Some phagolysosome inclusions were seen, but most lysosomes were found in macrophages in the subretinal spaces. The choriocapillaris was patent, and transmission electron microscopy showed reduplication of basement membrane. The underlying choroid looked normal.
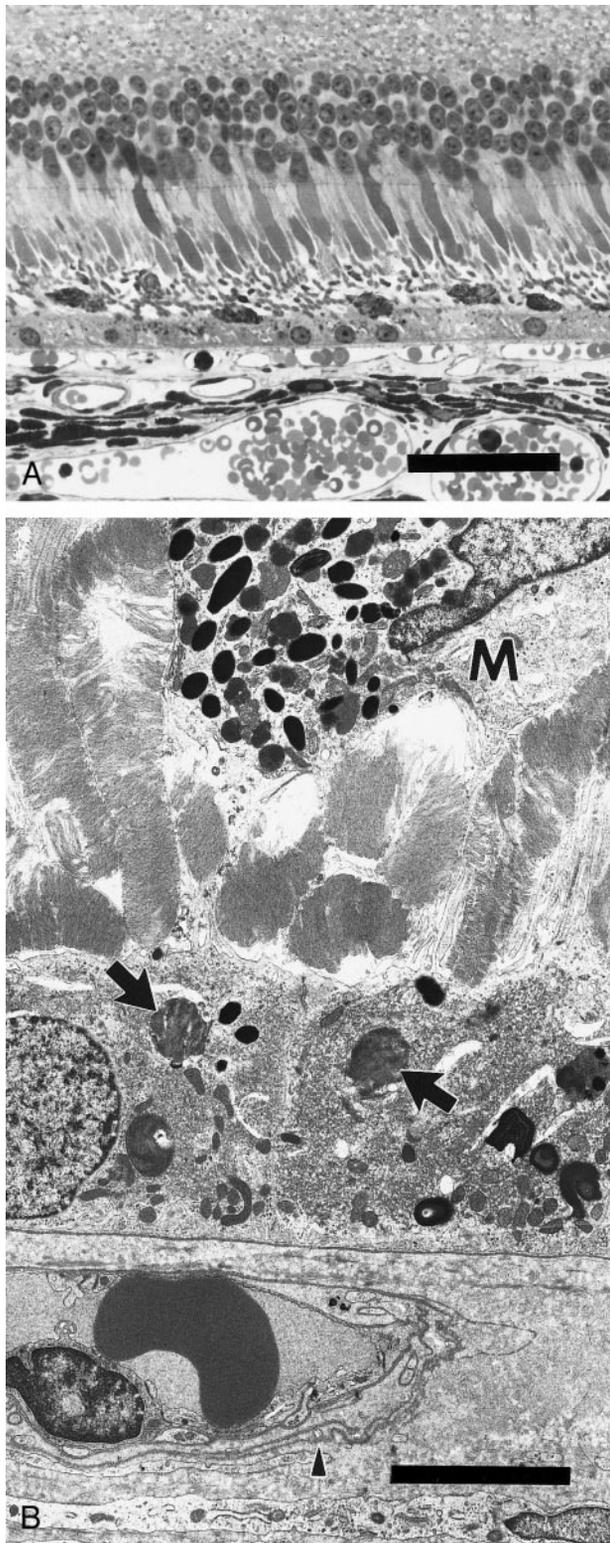
FIGURE 6.   (A) Light micrograph of PDT-treated normal monkey retina and choroid at 6 weeks after treatment. Inner segments appear normal, and outer segments are short and distorted. The RPE is hypopigmented and single layered, with pigment-laden cells overlying the RPE. The choriocapillaris and choroid are patent and appear fully recovered. Scale bar, 50 μm. (B) Transmission electron micrograph of normal retina and choroid 4 weeks after PDT. A pigment-laden cell (M) sits between disorganized outer segments on intact RPE. The RPE is lightly pigmented, has rudimentary basal infolding, elongated microvilli, and

ICG and BPD (Verteporfin) Angiography before PDT

Indocyanine green angiography demonstrated choroidal vessel perfusion at 5 to 7 seconds after injection of the dye (Fig. 7A). The CNV was visualized as a faint rim of hyperfluorescence during the later phase of the angiogram (Fig. 7B). The dye did not leak beyond the borders of CNV and fluorescence persisted up to 60 minutes after injection (longest follow-up after dye injection).

Angiography with Verteporfin provided visualization of small and large choroidal vessels at 5 to 7 seconds after intravenous injection of the dye (Fig. 7C). Dye fluorescence was seen in the retinal circulation 1 to 2 seconds after the choroidal circulation. Fluorescence in retinal vessels was bright at 10 to 15 seconds after the injection, then faded from the retinal circulation at approximately 10 minutes, and was not visible in the retinal vessels after 30 to 40 minutes. The CNV was visualized as a faint area of hyperfluorescence during the choroidal phase of the angiogram (Fig. 7C). The hyperfluorescence was bright at approximately 10 seconds after Verteporfin injection, and the dye did not leak beyond the borders of CNV (Fig. 7D) but fluorescence persisted for 60 minutes after injection (our longest follow-up after dye injection). BPD (Verteporfin) angiography provided better visualization of the experimental CNV than ICG angiography.

ICG and BPD (Verteporfin) Angiography after PDT

Indocyanine green angiography at 24 hours after PDT of CNV demonstrated hypofluorescence of the treated area with no perfusion of CNV, and late-phase angiography showed staining of the treatment spot. Indocyanine green angiography 5 weeks after PDT showed hypofluorescence of the treated area in the early phase of the angiogram (Fig. 8A), with perfusion of the retinal and large choroidal vessels. The late phase of the angiogram showed persistence of hypofluorescence in the treated area with some fluorescence in the area of treated CNV (Fig. 8B).

BPD (Verteporfin) angiography at 24 hours after PDT of CNV demonstrated hypofluorescence of the treated area with no perfusion of CNV in the early phase, and late-phase angiography showed staining of the irradiated CNV and the treatment spot. BPD (Verteporfin) angiography 5 weeks after PDT showed hypofluorescence of the treated area in the early phase of the angiogram, with perfusion of the retinal and large choroidal vessels and no perfusion in the CNV (Fig. 8C). The late phase of the angiogram showed persistence of hypofluorescence in the treated area with some staining of the treated CNV (Fig. 8D).

DISCUSSION

Photodynamic therapy using Verteporfin offers a potentially selective treatment for CNV due to AMD and other diseases. Previous studies have demonstrated that by adjusting the light

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several lysosomes containing outer segment material (*arrows*). The choriocapillaris is reperfused with reduplication of basement membrane (*arrowhead*). Scale bar, 5 μm.

FIGURE 7. (A) Early-phase (9 sec) ICG angiography of the monkey eye, showing dye in the small and large choroidal vessels and retinal vessels. No significant fluorescence is seen in the area of CNV. (B) Late-phase (37 min) ICG angiography of the monkey eye, showing some persistent dye in the large choroidal vessels and retinal vessels and a faint hyperfluorescent rim in the area of the experimental CNV. (C) Early-phase (13 sec) BPD (Verteporfin) angiography of the monkey eye, showing dye in the small and large choroidal vessels and retinal vessels and early hyperfluorescence of the experimental CNV. (D) Late-phase (33 min) BPD (Verteporfin) angiography of the monkey eye, showing some persistent dye in the retinal vessels and bright hyperfluorescence of the experimental CNV.
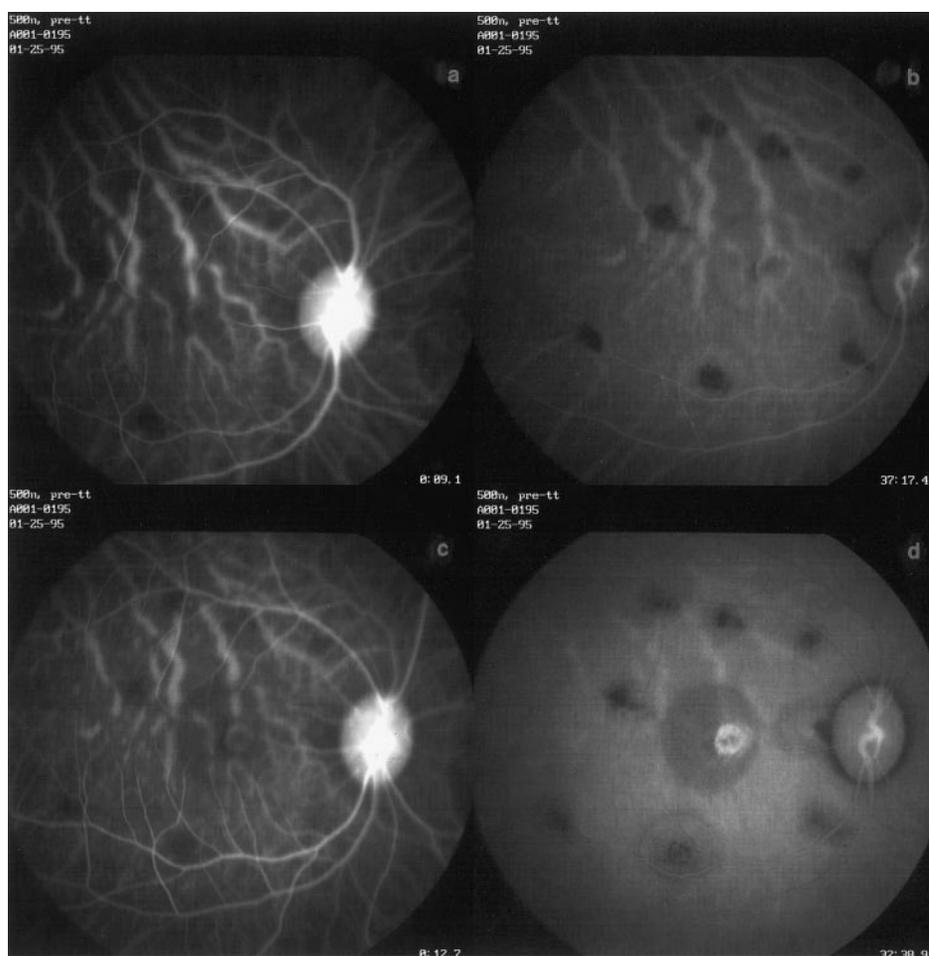
and dye doses, and the timing of irradiation after dye administration, vascular occlusion can be achieved in the choriocapillaris and CNV, with minimal disruption to the neurosensory retina. However, the long-term efficacy and the recovery of normal structures after PDT using Verteporfin have not been studied so far, and this is the first demonstration of the "longer-term" (4 to 7 weeks) effects of PDT on both CNV and normal retina and choroid.

The laser injury model of CNV in the primate is not ideal, because it is a wounding model and may not mimic the pathogenesis of CNV in AMD. Nevertheless, it provides a model of neovascularization originating from the choroid in the primate eye, with features common to clinical CNV, including angiographic and histologic features. More recently, studies have demonstrated that CNV in this model expresses growth factors and cell surface molecules such as vascular endothelial growth factor[14] and integrins,[15] which are also seen in CNV due to AMD. The model differs from clinical CNV in its tendency to regress, evidenced by a loss of angiographic leakage and envelopment by RPE cells.[12,16,] This may limit the ability of the model to assess long-term effects of CNV treatment. However, the changes that occur in the first few weeks after PDT assessed by FA, ICG, and BPD(Verteporfin) angiography, and light and electron microscopy may provide some useful information to predict and compare the effects in patients.

Fluorescein angiography early after PDT demonstrates hypofluorescence with perfusion of retinal vessels. BPD (Verteporfin) and ICG demonstrate deeper vasculature with most of the ICG fluorescence emanating from large choroidal vessels, and BPD (Verteporfin) fluorescence emanating from smaller choroidal vessels. The spectral characteristics of BPD (Verteporfin) are intermediate between fluorescein and ICG. Both ICG and BPD (Verteporfin) demonstrate hypofluorescence in the area treated with PDT, although larger choroidal vessels are perfused. This hypofluorescence with BPD (Verteporfin) and ICG angiography resolved to some extent with follow-up but was still evident at 5 to 8 weeks after PDT. The hypofluorescence may be caused by PDT-induced choriocapillaris occlusion or retinal pigment swelling leading to blocked fluorescence. Follow-up at 2 to 5 weeks after PDT of CNV demonstrated staining of the irradiated CNV by ICG and BPD (Verteporfin) angiography and may represent staining of the remaining fibrovascular tissue.

More intriguing are the findings of recovery of normal structures after PDT. The dye and light and dosing parameters of PDT can be adjusted to minimize effects on surrounding tissues, but the cells that are affected in the mildest treatments are the capillary endothelium and the RPE cells. Increased intensity of treatment results in increasing effects in the neurosensory retina, with disruption of the outer segments and pyknosis of the outer nuclear layer. At the parameters required for closure of CNV, one sees choriocapillaris occlusion, RPE cellular necrosis, and mild pyknosis of the outer nuclear layer; at 4 to 7 weeks after PDT these lesions show generalized recovery. The RPE repopulates the treated area, even when larger areas (4 mm) are treated. Although it has not been
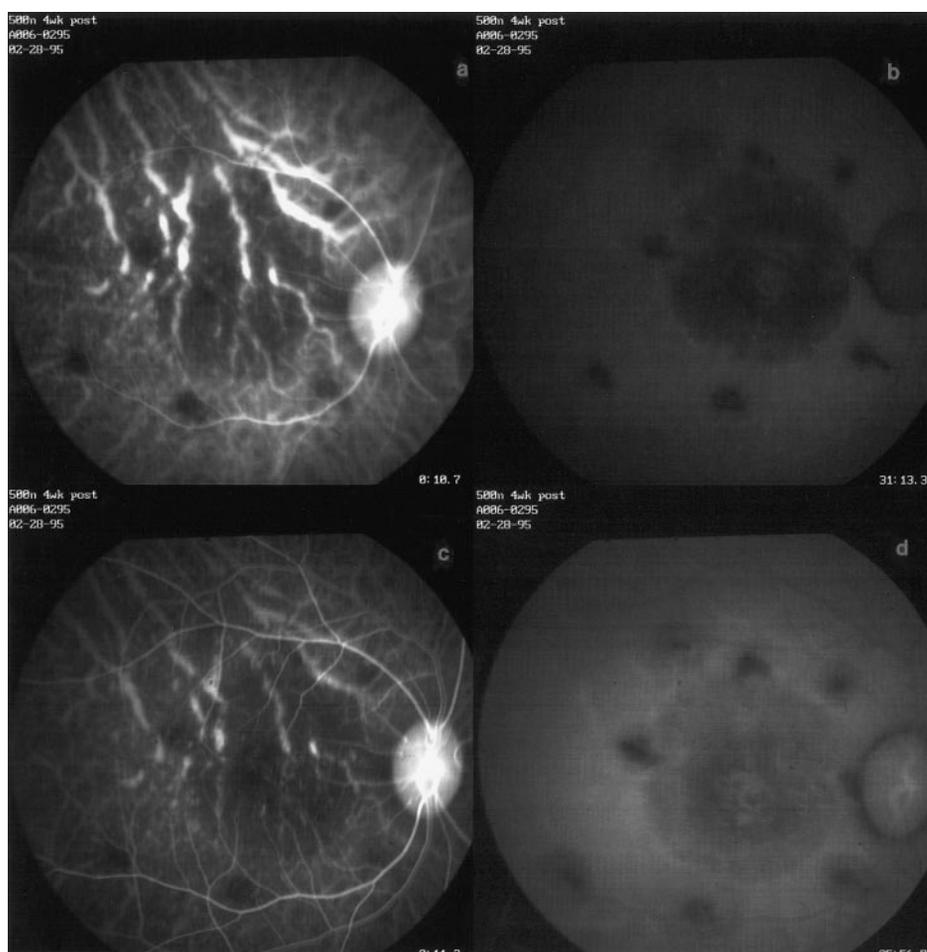
FIGURE 8. (A) Early-phase (10 sec) ICG angiography of the monkey eye taken 5 weeks after PDT for CNV. There is dye in the small and large choroidal vessels and retinal vessels and hypofluorescence of the PDT-treated area including the experimental CNV. (B) Late-phase (31 min) ICG angiography of the monkey eye taken 5 weeks after PDT for CNV. There is hypofluorescence of the PDT-treated area with some staining of the experimental CNV. (C) Early-phase (11 sec) BPD (Verteporfin) angiography of the monkey eye taken 5 weeks after PDT for CNV. There is dye in the small and large choroidal vessels and retinal vessels and hypofluorescence of the PDT-treated area including the experimental CNV. (D) Late-phase (36 min) BPD (Verteporfin) angiography of the monkey eye taken 5 weeks after PDT for CNV. There is hypofluorescence of the PDT-treated area and some staining of the treated experimental CNV.

demonstrated in this study whether this repopulation is due to proliferation or migration or both, Smiddy et al.[17] has shown that in adult primate retina proliferation of RPE can occur.

The RPE cells lining the treated areas in our study were found to be variably pigmented with basal infoldings and lysosomes, indicating some recovery of normal function. Similar recovery of the RPE has been described after surgical debridement of the RPE in primate eyes,[18] in porcine eyes,[19] and after laser injury to the RPE cells in rabbit eyes.[20] In our study, pigment-laden cells with phagocytic activity were seen overlying the RPE, and the significance of these is unknown. These cells have been previously reported in the literature and are proposed to be derived from histiocytes[21] or RPE cells.[22] In our study the choriocapillaris was reperfused and showed reduplication of basement membrane on electron microscopy, suggesting recanalization by migrating and proliferating endothelial cells after PDT. There also appeared to be some minimal loss of photoreceptors 4 to 7 weeks after PDT. The recovery of healthy RPE and choriocapillaris in the monkey eye may differ from the recovery in the elderly eye with AMD. The visual significance of PDT on normal structures will await the results of clinical trials.

The treatment parameters used in this study were based on previous studies of PDT using Verteporfin to treat experimental CNV.[3-5] The present study and the previous preclinical studies in monkeys were used to select the initial parameters used in the phase 1 and 2 clinical trials of PDT for CNV using Verteporfin. These included the Verteporfin dose of 6 or 12

mg/m^2, irradiance of 600 mW/cm^2, fluence of 50 to 150 J/cm^2, and irradiation applied 10 to 40 minutes after the start of Verteporfin infusion.[23-25] Subsequently the fluence was reduced to 50 J/cm^2 in the phase 3 trials when nonselective effects with damage to retinal vessels was observed at the highest fluence of 150 J/cm^2.[24,25]

Studies of PDT using Verteporfin in monkeys demonstrate that CNV can remain without angiographic leakage for 4 weeks and that the RPE and choriocapillaris show generalized recovery with preservation of the neurosensory retina over 4 to 7 weeks. The long-term efficacy and visual implications await the results of clinical trials.

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