Photodynamic Therapy of Pigmented Choroidal Melanomas

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Purpose. To evaluate the effectiveness of photodynamic therapy with chloroaluminum sulfonated phthalocyanine in the treatment of pigmented choroidal melanomas in a rabbit model.

Methods. Pigment containing B16F10 murine melanoma cells were implanted transclerally into the subchoroidal space of 28 immunosuppressed New Zealand albino rabbits. The animals were treated with daily injections of cyclosporine and were followed up until tumors at least 2 mm in height were detected by ultrasonography. Twenty-four hours after the intravenous injection of chloroaluminum sulfonated phthalocyanine (CASPc, 5 mg/kg), tumors were irradiated at 675 nm through an argon-pumped dye laser at estimated total light doses of 25 to 70 J/cm². Control animals were treated with light only or photosensitizer only. The animals were followed up for 4 1/2 to 8 weeks with regular fundus examinations.

Results. Twenty tumor-bearing rabbits were treated with light and dye. The tumor regressed in 12 animals. Five of these animals were followed up for at least 4 1/2 weeks and the other seven for 8 weeks after treatment. At light doses under 40 J/cm², tumor regrowth was observed in five animals within 10 days of treatment. In all control groups, the tumor-bearing eyes were filled with tumor cells by the third week after implantation. Histologic examination of tumors treated with photosensitizer and light revealed prominent vascular damage early after treatment that resulted in vascular occlusion. Tumor necrosis was evident within 24 hours of treatment.

Conclusions. Results suggest that photodynamic therapy may have a role in the treatment of pigmented choroidal melanomas. Invest Ophthalmol Vis Sci. 1995;36:871-878.

Photodynamic therapy (PDT) is based on the use of photosensitizers that generate oxygen radicals when activated by light of the appropriate wavelength.1-3 These dyes have been shown to concentrate preferentially within tumors. To date, the only photosensitizers that have been used in large clinical trials for the treatment of cancer are the hematoporphyrin derivatives. Although there is general agreement that hematoporphyrin derivative (HPD) is efficacious and safe as a therapeutic modality for a number of malignant tumors,4-7 PDT with this compound has been limited because of the associated skin photosensitivity of up to 2 months duration and the poor tissue penetration of light at 630 nm, the wavelength used to activate HPD.8

The development of second-generation photosensitizers associated with less skin phototoxicity and peak absorption wavelengths in the near infrared region, which markedly improves tissue penetration, has stimulated renewed interest in PDT. Among these are the phthalocyanines, well-characterized second-generation photosensitizers shown to be effective in the treatment of experimental tumor models.9-11 A few investigators have used phthalocyanine in the treatment of experimental iris and choroidal melanomas.12,13 However, the results obtained from these studies have limited clinical application because all the melanomas treated were amelanotic. Because the majority of human choroidal melanomas are pigmented, it is im-

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important to use an animal model with pigmented tumors to assess the efficacy of the phototherapeutic modality. Recently, we developed a pigmented experimental choroidal melanoma in our laboratory, and, with this model, we set out to evaluate the effect of PDT in the destruction of pigmented choroidal melanomas using chloroaluminum sulfonated phthalocyanine (CASPc; Porphyrin Products, Logan, UT).

METHODS

Cell Line

B16F10 murine melanoma cells (gift from Dr. R. Haining) were passaged in vitro in Dulbecco's minimal essential medium ( Gibco, Grand Island, NY) containing 10% fetal calf serum (Hyclone, Logan, UT). Two weeks before subchoroidal implantation was scheduled, 106 B16F10 cells in 0.1 ml phosphate-buffered saline were inoculated subcutaneously into the flank area of a C57BL/6 mouse. With this procedure, a palpable tumor usually is established within 10 to 14 days of implantation.

Tumor Model

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty-eight New Zealand albino female rabbits, each weighing 2.0 to 2.5 kg, were immunosuppressed with daily injections of cyclosporine-A (Sandimmunne; Sandoz, Basel, Switzerland) at a concentration of 20 mg/kg. All animals were anesthetized with an intramuscular injection of ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) and 5 mg/kg of xylazine hydrochloride (Haver-Mooney, Shawnee, KS). The tumor-bearing C57BL/6 mouse was killed immediately before implantation, and the tumor was dissected free and stored in cold 0.9% normal saline (Abbott Laboratories, North Chicago, IL) until its use.

The establishment of choroidal tumors was carried out as described. Briefly, the recipient animals were anesthetized as described above. A wire lid speculum was used to expose the recipient eye. An inferior quadrant was used, and a sclerotomy using a No. 64 blade was made (Beaver Surgical Products, Waltham, MA) into the subchoroidal space 5 mm from the limbus. A modified 22-gauge spinal needle with a blunt tip was used to deliver a 0.5-mm3 tumor fragment into the subchoroidal space, 6 mm posterior to the sclerotomy site. Proper tumor placement was verified with indirect ophthalmoscopy and a 20-D lens through a pupil dilated with 2.5% phenylephrine (Bausch and Lomb, Tampa, FL) and 1% tropicamide (Bausch and Lomb). To facilitate visualization, all tumors were implanted approximately 2 DD inferior to the optic nerve. The sclerotomy was closed with interrupted 8-0 nylon suture and the conjunctiva with 7-0 vicryl suture. Animals were evaluated daily by indirect ophthalmoscopy starting 7 days after implantation. All eyes implanted with fragments of B16F10 tumor yielded nodules 10 to 14 days after implantation. Tumor dimensions were assessed by B-scan ultrasonography using a Quantum 2000 ultrasound unit (Siemens, Issaquah, WA). Tumor base diameter at the time of entry into the experimental groups ranged from 2.5 mm to 7.0 mm (mean tumor diameter, 4.2 mm). The tumor height at the time of entry into the treatment groups ranged from 1.8 mm to 4.8 mm (mean height, 2.55 mm).

Photosensitizer

Chloroaluminum sulfonated phthalocyanine was obtained commercially in powder form and reconstituted in 0.9% normal saline immediately before administration. Five milligrams per kilogram was administered intravenously through an ear vein 24 hours before light exposure. The animals were housed in the dark for 1 week after administration of the dye to protect them against skin sensitivity and to ensure that all observed effects were caused by the laser light.

Laser Light Delivery System

Laser irradiations were delivered with a model 920 argon pumped dye laser (Coherent, Mountain View, CA). The laser was tuned to emit radiation at 675 nm. The laser beam was coupled to a 200 µm fiber optic coupler (Coherent) that was connected to a standard slit lamp delivery system. A 2.5-mm spot size was aligned to the slit lamp beam focal point to allow visual control during treatments. A low-power, 512-nm green light was used as the aiming beam during treatment. The desired irradiance at the retina was achieved by calculating the power output in relation to the area covered by the light after correction for the refractive power of the rabbit eye and the OGFA fundus contact lens (Ocular Instruments, Bellevue, WA). An irradiance of 150 mW/cm2 was used to deliver an estimated fluence of 80 J/cm2 and less per individual treatment spot. For fluences above 80 J/cm2, we used an irradiance of 165 mW/cm2. The higher irradiance was used to maintain individual spot exposure times below 7 minutes for the higher fluences. This was done to shorten overall treatment time as a convenience for the subject. Estimated fluences ranging from 25 to 70 J/cm2 were delivered by the application of overlapping spots until the tumor was completely treated. A half-spot size margin was given around the tumor.

Twenty-eight tumor-bearing animals were included in the treatment groups. Twenty eyes received
FIGURE 1. Ophthalmoscopic appearance of a tumor 3.1 mm in height before treatment.

FIGURE 2. (left) Ophthalmoscopic appearance of tumor growth in a treated eye.

FIGURE 3. (below) Ophthalmoscopic appearance of a choroidal melanoma. (below left) Before treatment. (below right) Eight weeks after photodynamic therapy with CASPc and a fluence at 40 J/cm². Note how the tumor nodule has been replaced by a pigmented chorioretinal scar. There is atrophy of the choroidal vessels in the areas of irradiation.
laser irradiation after dye administration. Control tumor-bearing animals received either laser irradiation but no dye (one at 40 J/cm², two at 60 J/cm², and two at 70 J/cm²) or dye administration without subsequent laser irradiation, or they were observed with no treatment. The laser power was checked before, during, and after treatment with a Field Master power meter (Coherent). Fundus photographs were taken 1 day before treatment; 1 hour, 1 day, and 1 week after treatment, and before sacrifice using a Canon CF-60ZA fundus camera (CF-60ZA; Canon, Lake Success, NY). Fluorescein angiography was performed at corresponding times with 0.1 mg/kg of 10% fluorescein (Akorn, Abita Springs, LA) injected through a marginal ear vein using the same camera.

Follow-up for the light plus dye group ranged from 4 ½ to 8 weeks after treatment. Follow-up for the control animals was limited to 3 weeks because of the rapid growth of the untreated tumor.

**Histopathology**

At selected time intervals after treatment, animals were killed with an intravenous injection of T-61 euthanasia solution (Hoechst–Roussel, Somerville, NJ; 0.3 ml/kg). Immediately after enucleation, the anterior segment was removed from each eye before fixation in 10% buffered formalin (Sigma, St. Louis, MO). After initial fixation, tumor regions were bisected and one side was processed for paraffin embedding and hematoxylin and eosin staining. The other half was refixed in 4% gluteraldehyde (Polysciences, War- rington, PA), dehydrated through alcohol, and embedded in epon. One-micron thick sections were stained with toluidine blue.

**RESULTS**

**Treatment Response**

Twenty-eight animals bearing tumors of different heights were used in our study. Figure 1 shows an example of a tumor 3.1 mm in height. Twenty tumor-bearing animals were treated with light and dye, and their responses are summarized in Table 1. Three animals were killed for histologic evaluation. The remaining 17 animals were followed up for 4 ½ to 8 weeks. Twelve demonstrated control of tumor growth. Tumor regrowth occurred in two animals treated with a fluence at 25 J/cm², one at 30 J/cm², and two at 35 J/cm². These tumors demonstrated growth within 10 days of treatment (Fig. 2). Tumor height did not appear to be responsible for the treatment failure because regrowth occurred in smaller tumors (Table 2). Tumor base diameter in the animals that demonstrated growth after treatment with light and dye ranged from 2.3 to 4.2 mm, with a mean of 2.7 mm. The base diameter in the treatment group ranged from 2.3 to 7.0 mm. All animals that received a total fluence greater than or equal to 40 J/cm² had control of tumor growth regardless of tumor height.

Lesions that showed complete regression demonstrated a typical clinical course. Immediately after treatment, there was a small serous retinal detachment, blanching of the tumor, and interruption of the surrounding choroidal vasculature. At 24 hours, the serous detachment was enlarged and the lesion was edematous and hemorrhagic. Over the ensuing week after PDT, the edema and serous detachment typically resolved. The tumor dimensions slowly diminished, leaving areas of chorioretinal atrophy. Eight weeks after treatment, the tumor nodules were replaced by gliotic scars that were encircled by an area of choroidal atrophy confined to the irradiated areas (Fig. 3).

Eight control animals were included in this study; five animals were treated with laser irradiation only. Two received dye only, and one received neither dye nor light. All control animals experienced tumor growth and had tumor occupying the vitreous cavity within 3 weeks of implantation of the tumor. Total retinal detachments developed as the tumors enlarged.

**TABLE 1. Comparison of Fluence to Treatment Outcome for Animals Treated With Dye and Light**

<table>
<thead>
<tr>
<th>Fluence (J/cm²)</th>
<th>Number of Animals</th>
<th>Tumor Arrest</th>
<th>Tumor Regrowth</th>
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<tr>
<td>25</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
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<td>2</td>
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</tr>
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<td>5</td>
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<td>5*</td>
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</tr>
<tr>
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</tr>
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<td>70</td>
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<td>1</td>
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<tr>
<td>Total</td>
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*Three animals were killed for histologic evaluation.*
TABLE 2. Comparison of Fluence to Tumor Height and Treatment Outcome

<table>
<thead>
<tr>
<th>Fluence J/cm²</th>
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<th>Outcome</th>
<th>Complication</th>
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<td>Arrest</td>
<td>Serous RD</td>
</tr>
<tr>
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</tr>
<tr>
<td>30</td>
<td>2.0</td>
<td>Regrowth</td>
<td>None</td>
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<tr>
<td>35</td>
<td>3.8</td>
<td>Arrest</td>
<td>Serous RD/subretinal hemorrhage/ conjunctival chemosis</td>
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<td>3.0</td>
<td>Arrest</td>
<td>Serous RD</td>
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<td>Regrowth</td>
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<td>Arrest</td>
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* Animal killed for histologic evaluation.

Travenous administration of 5 mg/kg of CASPc in the rabbit. Complications in the dye and light treatment group included conjunctival chemosis (two animals), subretinal hemorrhage (two animals), and serous retinal detachment (17 animals), which are comparable with other series12,13 (Table 2). In all cases, the complications resolved within 2 weeks of treatment.

**Histopathology**

Histopathologic examination of B16F10 melanoma in control eyes revealed cells with large nucleoli and variable amounts of pigmentation. Mitotic figures and numerous vascular channels were identified. Blood vessels exhibited intact endothelial cells with lumina containing red blood cells. Areas of tumor necrosis were present in larger tumors. Tumors receiving dye alone or laser irradiation alone exhibited histologic patterns similar to untreated controls (Fig. 5a). These specimens demonstrated no damage in the overlying retina at the light microscopic level.

Light microscopic examination after treatment with 40 J/cm² plus dye was performed at 1 hour, 4 hours, 12 hours, and 8 weeks after treatment. At 1 hour after treatment, dilated vessels filled with closely packed erythrocytes, and fibrin were found throughout the full thickness of the tumor (Fig. 5b). At 4 hours after treatment, similar vascular congestion was found. Increased evidence of cell damage, including condensation of the nuclear material of the tumor cells and the formation of cytoplasmic vacuoles, was observed. Twelve hours after PDT, the tumor cells had indistinct borders, and multiple pyknotic nuclei were evident. There was degeneration of the vascular channels that contained red blood cell ghosts and fibrin (Fig. 5c). At 8 weeks after treatment, mononuclear cells and pigment-laden macrophages were found in the zones of the regressed tumor (Fig. 5d). No viable tumor cells were observed. The retina overlying the treatment zone was completely disorganized. No damage was noted to the surrounding nonirradiated tissues.

**DISCUSSION**

Previous studies12,13,15 have suggested a potential role for PDT in the treatment of experimental iris and choroidal Greene Hamster amelanotic melanomas. Using 5 mg/kg of CASPc and estimated fluences ranging from 40 J/cm² to 70 J/cm², we were able to arrest growth of pigmented lesions ranging in height from 2.0 mm to 4.8 mm. All successfully treated animals were maintained for at least 30 days to establish treatment effect. The growth pattern associated with the pigmented B16F10 tumors is extremely aggressive; in untreated animals, the tumors are found to occupy the vitreous cavity within 3 weeks of implantation. In partially treated animals, tumor growth becomes obvious by ophthalmoscopy within 10 days of treatment.
The phthalocyanines are second-generation photosensitizers that have significant advantages over the hematoporphyrins. Low systemic toxicity, decreased skin photosensitivity, and a peak absorption spectrum higher in the infrared region are properties that make these dyes potentially useful for PDT. These characteristics are particularly important when treating pigmented lesions, because it has been shown that melanin absorbs short wavelength light to a greater extent than light at longer wavelengths. Therefore, treatment of pigmented lesions with CASPc is expected to be more efficient than treatment with HPD. Studies have demonstrated that light at 675 nm achieves 12% greater penetration in amelanotic tissues, such as retinoblastoma, and approximately 19% greater penetration in melanotic tissues, such as porcine brain, than light at 630 nm.

The mechanism of action of the phthalocyanines is thought to require oxygen. The CASPc molecule, after excitation to the triplet state by light of the appropriate wavelength, is thought to react with molecular oxygen to produce cytotoxic oxygen radicals. These radicals are thought to cause damage to vascular endothelium, resulting in ischemia, and to tumor cells directly by damaging cell organelles. After treatment with dye and light, fluorescein angiography demonstrates nonperfusion of the choroidal and tumor vessels in the irradiated tissues. Light microscopic examination of these tumors revealed vascular lumina packed with erythrocytes, platelets, and fibrin consistent with vascular closure. Our results are in agreement with previously published reports indicating that vascular changes are the predominant early findings after PDT and that they play an important role in arresting tumor growth.

Our results suggest that pigmentation does not appear to have a negative impact on the effectiveness of PDT using CASPc in this model. In fact, some authors have proposed the possibility that pigmentation may actually have a beneficial effect on therapy because of melanin's ability to absorb light energy and to heat the tissues. Hyperthermia has been shown to be induced by therapeutic laser radiation used for PDT in human and animal studies. It also has been shown to potentiate the effects of PDT and radiotherapy in the treatment of different tumors. Thermal effects on tissues with wavelengths in the red region of visible light have been reported to be significant at irradiances above 100 mW/cm², and, with the irradiances of 150 mW/cm² and 165 mW/cm² used in our study, we expected some thermal effects to be present. However, these effects alone are ineffective in the destruction of pigmented choroidal tumors, as demonstrated in our control animals treated with light only.

**FIGURE 4.** Angiographic features of a choroidal melanoma before and after PDT. (a) Fundus photograph of a choroidal melanoma with a subretinal hemorrhage before treatment. (b) Late frames of the angiogram show hyperfluorescence of the lesion before treatment. There is blocking of choroidal fluorescence inferiorly, which corresponds to the area of hemorrhage. (c) Fundus photograph of the same tumor 1 hour after treatment with 40 J/cm² plus dye. There is blanching of the tumor with an overlying serous retinal detachment. (d) Fluorescein angiogram 1 hour after treatment with 40 J/cm² plus dye. There is marked hypofluorescence, corresponding to the area of treatment.
Photodynamic Therapy of Pigmented Choroidal Melanomas

Our study suggests that PDT using CASPc may have a role in the treatment of pigmented choroidal melanomas. Although there is a general concern that light penetration might be the limiting factor when treating pigmented choroidal tumors, it appears that second-generation photosensitizers with peak absorption wavelengths in the infrared region may provide adequate destruction of small to medium tumors. Experiments currently under way will evaluate the maximal tumor height amenable to this modality.

**Key Words**
photodynamic therapy, choroidal melanoma, photosensitizer, rabbit model, pigmented melanomas

**Acknowledgment**
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**References**