Photodynamic Therapy of Ocular Melanoma With Bis Silicon 2,3-Naphthalocyanine in a Rabbit Model

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Purpose. To investigate bis (tri-n-hexylsiloxy) silicon 2,3-naphthalocyanine (SINc; 0.5 mg/kg) for photodynamic therapy of an experimental ocular melanoma in pigmented rabbits.

Methods. SINc was dissolved in canola oil by heating, emulsified with Tween 80, and administered by ear vein. Pharmacokinetics were studied in frozen tumor sections by fluorescence microscopy using a charge coupled device, camera-based, low-light detection system with digital image processing at 1 and 24 hours. A Ti:sapphire laser and a microlens were used to deliver the light (770 nm; 40 mW/cm²; 20 J/cm²). A control rabbit received light without SINc.

Results. Localization studies of SINc showed intravascular distribution shifting to a tumor stromal and perivascular distribution 24 hours after treatment. Tissue thermal damage after irradiation was minimal in the control. Exudative retinal detachments were not observed. Tumor destruction was observed, with sharp demarcation to a depth of 3.5 mm.

Conclusions. Tumor light penetration was good at 770 nm, and thermal effects from the exciting light alone were minimal. Photodynamic therapy with SINc resulted in localized tumor destruction reflecting the light beam path without damage to adjacent tissue or intraocular complications. Invest Ophthalmol Vis Sci. 1995;36:2476-2481.

Photodynamic therapy (PDT) is a nonthermal technique that may be useful for the treatment of ocular tumors. This technique leaves adjacent structures largely intact because of the differential uptake of photosensitizers by tumors.1-7 Usually, PDT requires systemic or topical administration of the photosensitizer. After tissue or tumor uptake of the photosensitizer, absorption of light by the photosensitizer converts it to an excited singlet state, which may decay to the ground state (fluorescence) or undergo intersystem crossing to the triplet state. The longer-lived triplet state may decay to the ground state (phosphorescence) or interact with the substrate or with oxygen directly. Interaction of the triplet state with the substrate produces substrate and photosensitizer radicals that may then interact with oxygen to produce reactive oxygen species (hydroxyl radicals, peroxides, and superoxides; type 1 photosensitizer reaction). Interaction of the triplet state with oxygen produces singlet oxygen by a spin-state transition (type 2 photosensitizer reaction). The type 2 reaction is responsible for most of the damage during PDT, whereas type 1 reactions may have a minor role during PDT.9 Depending on the type of photosensitizer used, damage to the vasculature and direct cytotoxic effects are responsible for tissue damage in varying degrees.10-14 The potential for photoreceptor absorption and damage is significant at 633 nm, and significant thermal effects from melanin absorption may occur at the irradiance levels necessary to perform PDT.15,16 Photosensitizers with longer wavelength absorption maxima could have advantages over those with shorter wavelength maxima. One advantage is increased transscleral transmission of laser light.17 This would allow transscleral deliv-
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FIGURE 1. Structure of the photosensitizer, bis (tri-n-hexylsiloxy) silicon 2,3-naphthalocyanine (SINc).

ery of light to augment transpupillary treatment, maximizing tumor treatment depth. Other possible advantages of longer wavelength maxima are decreased photoreceptor interaction and decreased thermal effects from melanin absorption. In this study we have studied one such photosensitizer, bis (tri-n-hexylsiloxy) silicon 2,3-naphthalocyanine (SINc) with an absorption maxima of 770 nm (Fig. 1).

MATERIALS AND METHODS

All experiments adhered to institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty pigmented rabbits (1.5 to 2 kg) were used in all experiments. Before all treatments, the animals were anesthetized with an intramuscular injection of 0.75 ml of a 2:1 solution of ketamine hydrochloride (100 mg/ml) and xylazine hydrochloride (20 mg/ml). All animals were examined with a slit lamp before sacrifice by a lateral ear vein injection of Eutha-6.

Tumor Implantation

Greene hamster melanoma was grown by serial anterior chamber passage in five donor rabbits. Donor animals were killed, and tumor fragments were implanted in the subchoroidal space of an additional animal using a modification of a previously reported technique. Briefly, with the aid of an operating microscope, a 7/8 thickness scleral cutdown was made in the supranasal quadrant 7 mm posterior and parallel to the limbus of the eye. A posterior scleral pocket then developed. At the terminus of this pocket, the suprachoroid was entered with a cyclodialysis spatula, and 0.25-mm tumor fragments were implanted with the aid of a 22-gauge catheter. The scleral flap and conjunctiva were then closed with 6-0 vicryl suture material.

Photosensitizer Preparation

Bis (tri-n-hexylsiloxy) silicon 2,3-naphthalocyanine (SINc) was dissolved in five parts by weight canola oil by heating. Five parts by weight Tween 80 (Aldrich Chemical, Milwaukee, WI) was added to the canola oil mixture after cooling. The resultant mixture was emulsified with 95 parts water, and a dosage of 0.5 mg/kg was administered by lateral ear vein after sedation.

Photosensitizer Localization Experiments

Photosensitizer localization studies were performed at 1 (three rabbits) and 24 hours (three rabbits) after the intravenous administration of SINc. These tissues also served as the SINc-treated, nonirradiated tumor control tissue. After microsurgical resection, the specimens were placed in boats containing OCT embedding medium (Miles, Elkhart, IN), rapidly frozen with crushed dry ice, stored at —80°C, and handled in low diffuse light. Six-micrometer thick serial sections were prepared for fluorescence studies (Cryostat microscope; AO Reichert, Buffalo, NY). A Zeiss Axiosvert 10 inverted microscope (Zeiss, Oberkochen, Germany) was used with a x10 objective (Zeiss Achrostigma, NA = 0.3) to visualize phase contrast (phase 1) and fluorescence images of tissue frozen sections. Light from a 100-W mercury lamp filtered through interference filters (either 365 nm or 400 nm bandcenter, 30 nm full width half-maximum height) provided the excitation source. The excitation light was reflected onto the sample using dichroic filters (either FT395 or FT580; Zeiss), and the emission was isolated with a 615-nm long-pass filter. The images were recorded with a cooled, slow-scan charge coupled device camera (576 × 384 pixel; 16-bits per pixel dynamic range) (Princeton Instruments, Trenton, NJ) interfaced to a Macintosh computer. Instrument control and image processing were carried out with IPLab software (Signal Analytics Corp.). A UniBlitz shutter and driver (model T132) were used to synchronize the charge coupled device camera with the mercury lamp to minimize sample photophotobleaching. Typical exposure times were 1 second for fluorescence images. A custom-built programmable stage was used to control sample x–y motion with 0.1 µm precision. Image acquisition and camera–stage control were carried out by a Macintosh Ilfx computer (Apple, Cupertino, CA) with appropriate software.

To estimate light distribution, background images

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were acquired from blank slides with identical parameters (i.e., filters, exposure times). To correct for nonuniform illumination, all fluorescence images were normalized by the following algorithm to correct for nonuniform illumination:

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\text{Normalized fluorescence image} = \frac{\text{fluorescence} - \text{background}}{\text{background}}
\]

Both fluorescence and background images were corrected for DC (dark) noise generated during the exposure period.

**Photodynamic Therapy**

A drop of proparacaine hydrochloride 0.5% (Alcon, Humacao, PR) was placed on the eye and was followed by a wire lid speculum. The 770-nm light was provided by an argon ion pumped Ti:sapphire laser (Coherent, Palo Alto, CA). The wavelength of the light was verified to ±1 nm with a Hartridge Reversion Spectroscope (Ealing Electro-Optics, Holliston, MA). Light was transmitted with a Model 316 fiberoptic coupler into a 1000-μm quartz optical fiber (Mitsubishi Cable Industries, Fort Lee, NJ). The resultant beam was divergent. Laser dosimetry was chosen based on previous studies of PDT of iris melanomas and from previous studies of retinal pigment epithelium and choroid using 675 nm of light incident on the cornea.

Four SINc-treated rabbits with tumor-bearing eyes were irradiated 24 hours after the administration of photosensitizer. Laser light was delivered to the tumor using a microlens at 40 mW/cm²; the total light dose was 20 J/cm². Continuous wave power was measured with a power meter (Spectra-Physics [Mountain View, CA] 404) before and after treatment.

Four SINc-treated, nontumor-bearing eyes (contralateral eyes of successful implants) were left unirradiated for toxicity studies. Thermal controls consisted of one eye with a successful tumor implant that received laser irradiation but no photosensitizer and one unsuccessful tumor implant eye that received laser without SINc. One successful tumor was examined without SINc or laser for baseline necrosis.

Immediately after tumor resection, tissue was fixed in either buffered 10% formaldehyde or fresh half-strength Karnovsky’s. After fixation, the eyes were inspected and subsectioned using a dissecting microscope to isolate the tissue of interest for embedding.

Samples for paraffin sections were dehydrated in a series of graded alcohols, cleared in Histoclear (National Diagnostics, Atlanta, GA), and embedded in paraffin. Six-micron serial sections were cut, cleared in Histoclear, stained with hematoxylin and eosin, and covered with cover slips. All sections were examined, and representative sections were photographed (T-50 or T-Max 100; Kodak, Rochester, NY).

**FIGURE 2.** (A) Bright-field examination of a frozen tumor section 24 hours after intravenous injection of SINc (0.5 mg/kg). Note the lack of tissue autofluorescence in the tumor. Original magnification, ×100. (B) Fluorescence of a frozen tumor section 24 hours after intravenous injection of SINc (0.5 mg/kg). Fluorescence is located primarily in perivascular positions within the tumor. Original magnification, ×100.
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FIGURE 3. Tumor tissue section 3 days after photodynamic therapy. The tumor was treated with laser light (20 J/cm²; 40 mW/cm²; 770 nm) 24 hours after the intravenous injection of SINc (0.5 mg/kg) and irradiation. There is sharp demarcation between damaged and undamaged areas of the tumor, reflecting the laser light beam path. Hematoxylin and eosin; original magnification, ×40.

RESULTS

Photosensitizer localization studies showed a primarily intravascular distribution of SINc 1 hour after injection. Within 24 hours, the SINc underwent a redistribution to perivascular and tumor stromal positions (Fig. 2). Before the animals were killed, all eyes were quiet, and no animal required medication for discomfort. One animal with a successful tumor implant died of presumed Pasteurella pneumonitis 24 hours after the administration of SINc.

After enucleation and fixation, all eyes underwent gross pathologic examination with a dissecting microscope. In eyes with successful tumor implants treated with PDT, exudative retinal detachments were not seen. One tumor had a small area of overlying vitreous hemorrhage. Histopathologic analysis of PDT-treated tumors revealed a sharp demarcation between irradiated and nonirradiated areas reflecting a laser beam path (Fig. 3). Tumor destruction was evident in treated areas to a depth of at least 3.5 mm. The transition zone was remarkable when viewed at higher magnification (Fig. 4). Tumor cells within the necrotic treated area exhibited pyknosis (nuclear shrinkage), karyorrhexis (nuclear fragmentation), and eosinophilia without nuclear detail as manifestations of irreversible cell death. There was also early disruption of stomal integrity. Mitotic figures were not seen in the treated area but were evident in the untreated areas. All control tumor tissues (SINc treated, nonirradiated; non-SINc-treated, nonirradiated; non-SINc-treated, irradiated) showed a monotonous collection of pleomorphic cells with an immature reticular vascular system and abundant mitotic figures. The tumor necrosis present in the PDT-treated tissues was not observed in control tissue. Histopathologic studies of the SINc-treated, nonirradiated, contralateral eyes of successful tumor implants (nontumor bearing) were unremarkable at the light microscopic level. The final thermal control, an irradiated, non-SINc-treated, nontumor-bearing eye, did not demonstrate thermal effects.

DISCUSSION

Selective destruction of this experimental ocular melanoma to a depth of 3.5 mm was effected in this study. The selectivity is attributed to the preferential uptake and retention of the photosensitizer by the tumor. Demarcation zones between tumor necrosis and normal tumor were sharp, reflecting the laser beam path. Tissues, such as the ciliary body, which selectively retained the SINc, could have been injured by scattered radiation. This was not seen in our study because tumors were illuminated directly, and, as a result, relatively little radiation was scattered. If transscleral illumination had been used as a second treatment to increase treatment depth, laser light could have been scattered from the edges of the tumor, and this could have increased the risk to these tissues.

Photodynamic therapy-induced exudative retinal detachments have been reported after direct retinal illumination with laser light. These detachments have occurred after irradiation at 550 nm (120 mW/cm²; 26.6 J/cm²) and 675 nm (120 mW/cm²; 43 J/cm²). These exudative detachments are thought to be secondary to transient increases in choroidal vascular permeability. Exudative retinal detachments were not observed in this study.

Although the phthalocyanines generally are stable...
and probably have low systemic toxicity, there were some difficulties associated with the SINc. The most difficult problem is associated with its low solubility. Because of this, SINc could be dissolved only by heating with sonication, and it required a relatively high-volume emulsion delivery system. If a proportionate amount of the emulsion were delivered to humans, more than 1 l of hypotonic intravenous fluid would be required. The potential health risk to the patient resulting from this could be unacceptable. Other methods of delivery may be possible, and this photosensitizer may be useful in topical preparations.

In addition to problems associated with the solubility of SINc, another possible area of improvement is the absorption maxima. It may be possible to prepare an analog of SINc that is more soluble and has a wavelength maxima in the diode laser range (790 to 830 nm). This shift into the near infrared would increase tumor photon penetration and transscleral transmission efficiency, allowing lower irradiances and potentially decreased interaction with melanin and photoreceptors. One final practical restriction to the clinical application of SINc is the cost of preinvestigative new drug toxicology studies.

Key Words
laser, melanoma, ocular tumor, photodynamic therapy

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References
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