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Photodynamic Therapy (PDT) of the Ciliary Body With Silicon Naphthalocyanine (SINc) in Rabbits

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Background and Objective: **To investigate silicone naphthalocyanine (SINc; 0.5 mg/kg) for photodynamic therapy (PDT) of the ciliary body in pigmented rabbits.**
Study Design/Materials and Methods: **SINc was dissolved in canola oil by heating, emulsified with Tween-80, and given by ear vein. Pharmacokinetics were studied in frozen sections by fluorescence microscopy using a CCD camera-based, low light detection system with digital image processing at 1 hr and 24 hr (12 rabbits, 24 eyes total). A Ti:Sapphire laser delivered light at 770 nm by contact fiberoptic (1,000 μm; 80 mW/cm^2;20,40 and 80 J/cm^2). Controls (5 rabbits), received laser light at 770 nm without SINc. For comparison, eyes received continuous wave Nd:YAG laser by fiberoptic contact (0.8–1.2 J).**
Results: **Localization studies showed intravascular distribution shifting to a ciliary body distribution at 24 hr. PDT at 1 hr and 24 hr postinjection showed a more selective destruction of the ciliary body at 24 hr. Ciliary processes treated at 24 hr showed infarction and marked edema with sparing of iris. Tissue thermal damage was minimal in PDT controls. Eyes treated with the Nd:YAG laser exhibited full-thickness thermal necrosis of iris, ciliary processes, and a fibrinous iridocyclitis. In contrast, eyes treated by PDT were quiet with thrombosis of superficial blood vessels.**
Conclusion: **Tissue photon penetration is good at 770 nm and thermal effects from the exciting laser alone were minimal. The ciliary processes of pigmented rabbits exhibit a selective retention of SINc and on that basis can be selectively destroyed with a minimum on thermal damage to nontarget tissues.**

Key words: cyclodestructive surgery, glaucoma, photodynamic therapy

INTRODUCTION

Transscleral laser cyclophotocoagulation is a cyclodestructive glaucoma surgery used in the treatment of glaucoma with poor surgical and/or visual prognosis. It utilizes near-infrared lasers with relatively nonspecific thermal effects to transsclerally destroy the aqueous secretory structures. Although this technique has a moderate

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level of success, a significant amount of pain, inflammation, visual loss, and hypotony is associated with its use [1–5]. The ideal cyclodestructive procedure would destroy the aqueous secretory structures with minimal contiguous tissue damage. Photodynamic therapy (PDT) is a nonthermal technique that may be useful for cyclodestructive surgery because of the differential uptake of photochemicals by vascular tissues such as the ciliary body [6]. PDT requires systemic or topical administration of a photochemical. After tissue or tumor uptake of the photochemical, absorption of light by this molecule may convert it to an excited singlet state that may decay to the ground state (fluorescence) or undergo intersystem crossover to the triplet state. This longer lived triplet state may decay to the ground state (phosphorescence) or interact with substrate or oxygen directly. Interaction with substrate produces substrate and photosensitizer radicals, which may then interact with oxygen to produce reactive oxygen species (hydroxyl radicals, peroxides, and superoxides; Type I reaction). The triplet state may interact directly with oxygen producing singlet oxygen via a spin-state transition (Type II photochemical reaction) [7]. The type II mechanism is responsible for most of the damage during PDT, whereas Type I reactions may have a minor role during PDT [8]. Depending on the type of photochemical used, damage to the vascular supply and direct cytotoxic effects are responsible for tissue damage in varying degrees [9–13].

Hematoporphyrin derivative (HPD) is one photochemical that has been studied previously in the PDT mediated destruction of the rabbit ciliary body [14]. The wavelength that corresponds with the absorption peak for HPD penetrates poorly through sclera, requiring contact fiber optics to increase transscleral transmission efficiency [15]. The potential for photoreceptor absorption and damage is significant at 633 nm and significant thermal effects from melanin absorption may occur at irradiance levels necessary to perform PDT [6]. Photochemicals with longer wavelength absorption maxima would have theoretical advantages in terms of transscleral transmission of laser light, photoreceptor interaction, and melanin absorption. To increase transscleral transmission and decrease photoreceptor and melanin absorption, a photochemical would need a longer absorption maxima. In this current study we have studied one such photochemical, silicon naphthalocyanine [16] with an absorption maxima of 770 nm.

MATERIALS AND METHODS

All experiments adhered to institutional guidelines and the ARVO Resolution on the Use of Animals in Research. Twenty-six pigmented rabbits (1.5–2.0 kg) were used in all experiments. Prior to all laser treatments, 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 prior to sacrifice with an lateral ear vein injection of Eutha-6 after induction of anesthesia (above).

Photochemical Preparation

SINc was synthesized and purified by Dr. Malcolm Kenney. It was dissolved in five parts by weight canola oil by heating. Five parts by weight Tween-80® was added to the canola oil mixture after cooling. The resultant mixture was then emulsified with 95 parts water and given by lateral ear vein after sedation at a dosage of 0.5 mg/kg.

Photochemical Localization Experiments

Photochemical localization studies were performed at 1 hr (six rabbits) and 24 hr (six rabbits) after intravenous administration. 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. Serial sections (6 μm thick) were prepared for fluorescence studies (Cryostat microtome, AO Reichert, Buffalo, NY). A Zeiss Axiovert 10 inverted microscope was used with a 10× objective (Zeiss Achrostigma, N.A. = 0.3) to visualize phase contrast (phase 1) and fluorescence images of tissue frozen sections. A 100 W mercury lamp filtered through interference filters (either 365 nm or 400 nm bandcenter, 30 nm FWHM) provided the excitation source. 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. All images were recorded using a cooled, slow-scan CCD camera (576 × 384 pixel; 16-bits per pixel dynamic range) system (Princeton Instruments, Trenton, NJ) interfaced to a Macintosh computer. Instrument control and image processing were performed with IPlab software (Signal Analytics Corp.). A UniBlitz shutter and driver (model T132) were used to synchronize the CCD-camera with the mercury lamp in order to minimize sample photo bleaching. Typical exposure

times were 1 sec for fluorescence images. A custom-built programmable stage controls sample x-y motion with 0.1 μm precision. Image acquisition and camera/stage control are performed by a Macintosh IIfx computer with appropriate software.

In order to estimate light distribution, background images were acquired from blank slides with identical parameters (i.e., filters, exposure times). All fluorescence images were normalized by the following algorithm to correct for nonuniform illumination: Normalized fluorescence Image = (fluorescence − Background) / Background. Both fluorescence and background images were corrected for DC (dark) noise contributed during the exposure time.

Transscleral Irradiation Experiments

A drop of proparacaine hydrochloride 0.5% (Alcon, Humacao, PR) was placed on the eye followed by a wire lid speculum. An argon ion pumped titanium:sapphire laser (Coherent; Palo Alto, CA) was used in all experiments. Wavelength was verified to +/− 1 nm by using a Hartridge Reversion Spectroscope (Ealing Electro-optics, Holliston, MA). Light was transmitted with a Model 316 fiber optic coupler into a 1,000-μm quartz optical fiber (Mitsubishi Cable industries, Fort Lee, NJ), producing a divergent beam of light. Laser dosimetry was chosen based on previous studies of PDT of iris melanomas [17] and expected iris pigment epithelium attenuation estimated from previous studies of retinal pigment epithelium and choroid for 675 nm light incident on the cornea [18]. An irradiance of 80 mW/cm^2 was used to deliver total dosages of 20, 40, or 80 J/Cm^2. The laser irradiation was monitored with a Spectra-Physics 404 power meter before and after treatment.

A total of 14 rabbits (28 eyes) were included in the laser-treated groups. Ten eyes (five in each group) received laser irradiation after SINc injection. Laser energy was delivered by fiber optic contact at the rabbit limbus at one (right eye) and 24 hr (left eye) after injection of SINc. Thermal controls received laser irradiation (80 mW/cm^2) at 770 nm (20, 40, or 80 J/cm^2) but no photochemical (4 eyes), and four eyes underwent fiber optic contact (600 μm; quartz optical fiber; Mitsubishi Cable Industries) transscleral Nd:YAG cyclophotocoagulation at 6,8,10,12 W with an exposure time of 100 ms (0.6–1.2 J).

Immediately following sacrifice and resection of the anterior segment, the tissue was fixed in either buffered 10% formaldehyde or fresh 1/2 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, and embedded in paraffin. Serial sections (6 μm) were cut, cleared in histoclear, stained with hematoxylin and eosin, and covered with coverslips. The tissue studied in plastic sections was transferred from 0.1 M cacodylate buffer and postfixed in 1% osmium tetra oxide in 0.1 M cacodylate buffer for 1 hr, rinsed in double distilled water, and stained with Richardson's stain (1% methylene blue and 1% Borax mixed 50:50).

All sections were examined and representative sections were photographed (T-50 or T-Max 100; Kodak, Rochester, NY) with an Olympus photo microscope using Zeiss optics.

RESULTS

Photochemical localization studies showed a primarily intravascular distribution of SINc at 1 hr. After 24 hr, the SINc underwent a redistribution into the substantia propria of the rabbit ciliary body (Fig. 1a,b).

Prior to sacrifice, eyes that had undergone PDT at 24 hr postinjection exhibited minimal cell or flair by slit lamp examination. Eyes that had undergone treatment with the Nd:YAG laser exhibited a marked inflammatory response, which varied from conjunctival injection and anterior chamber inflammatory cell and flair (Tyndall effect secondary to increased aqueous proteins) to a fibrinous iridocyclitis.

On gross pathologic analysis, alteration of the ciliary processes was noted for both the 40 and 80 J/cm^2 light doses. The ciliary processes appeared to have an edematous appearance on examination. Histopathologic analysis was markedly different between the Nd:YAG and PDT treated groups. Irradiation of rabbit ciliary body at 24 hr postinjection of SINc produced infarction and disorganization of the ciliary body. There were minimal thermal effects and these were primarily limited to vacuole formation in pigmented epithelial cells of the iris > ciliary body. The stroma of the cornea/sclera and iris were largely unaffected (Fig. 2).

Four control eyes received only laser treatment at 770 nm without photosenitizer injection

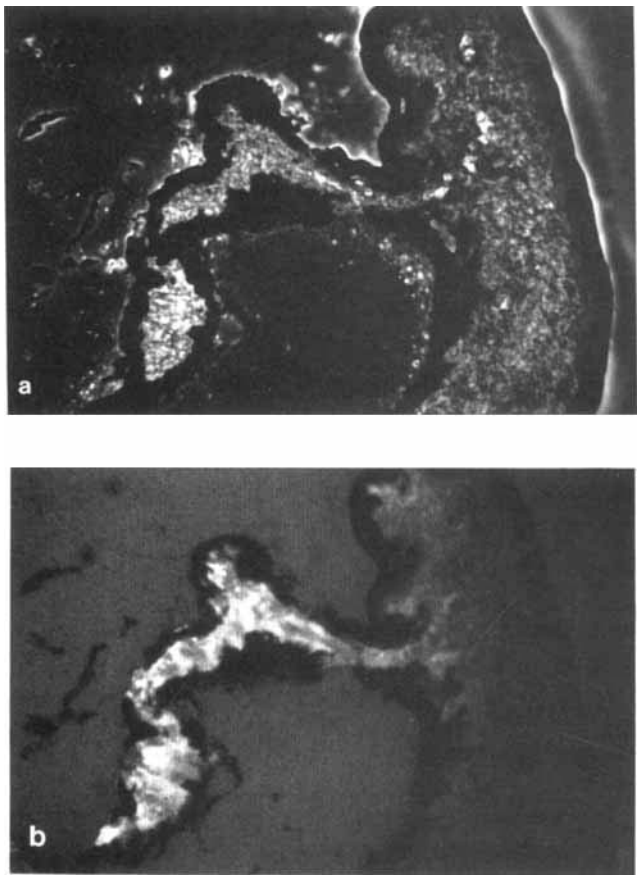

Fig. 1. (**a**) Brightfield examination of a frozen tissue section 24 hr after intravenous injection of (0.5 mg/kg) SINc. Note the lack of tissue autofluorescence in the ciliary body (original magnification = 100×). (**b**) Flourescence of a frozen tissue section 24 hr after intravenous injection of (0.5 mg/kg) SINc. The fluorescence is primarily located in the substance of the ciliary body (original magnification = 100×).

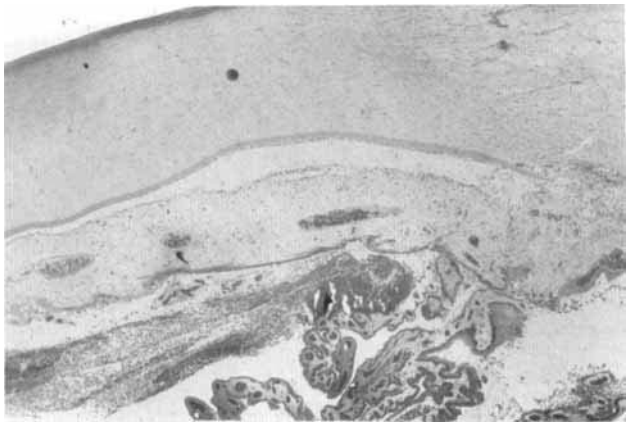

Fig. 2. Plastic tissue section 24 hr after intravenous injection of (0.5 mg/kg) SINc and irradiation (60 J/cm^2; 80 mW/cm^2) at 770 nm. Infarction and disorganization are seen in the ciliary body. There are minimal thermal effects that are primarily limited to vacuole formation in pigmented epithelial cells of the iris > ciliary body. The stroma of the cornea/sclera and iris are largely unaffected. Richardson's stain (original magnification = 40×).

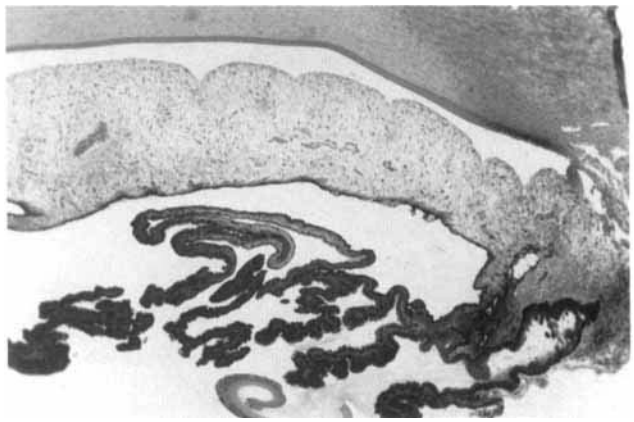

Fig. 3. Rabbit ciliary body after irradiation without photochemical (770 nm; 80 mW/cm^2; 80 J/cm^2). Richardson's stain, slightly overstained; original magnification = 40×. The extensive disorganization noted in the 24-hr-treated PDT group is not seen.

(irradiance, 80 mW/cm^2; exposure 20, 60, and 80 J/cm^2). Histologic analysis of this tissue did show evidence of background thermal damage consistent with melanin photon uptake. The posterior pigmented layer of the iris exhibited more signs of alteration that the pigmented ciliary body epithelium. The extreme internal disruption, swelling, and vacuolization of the ciliary processes was not seen in any tissue thermal controls (Fig. 3). Both the PDT treated tissue and the thermal controls compared favorably to the largely thermal process of the Nd:YAG laser (0.8 J). This group (Fig. 4) showed a much larger lesion with full thickness thermal necrosis of the iris and ciliary body. In addition, thermal injury to overlying sclera with influx of inflammatory cell is also present.

DISCUSSION

The principle of selective destruction of the ciliary processes based on the preferential uptake and retention of a photochemical by the ciliary body was previously demonstrated using Photofrin® II (P II) [14]. Despite the use of fiberoptics to increase transmission, photochemicals with absorption maxima in the visible such as P II may have limitations based on a potential for injury to photoreceptors or thermal effects secondary to in-

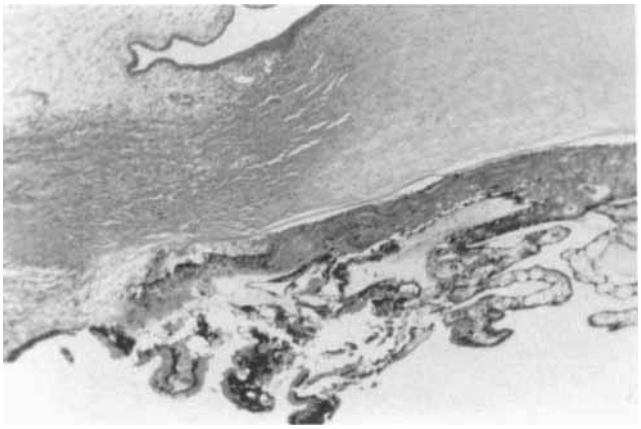

Fig. 4. Tissue treated with the Nd:YAG laser (0.1s; 8W) exhibited full thickness thermal necrosis of iris and ciliary processes (original magnification = 40×).

creased photon uptake in melanin containing tissue.

In this pilot study we investigated SINc with an absorption maxima (770 nm) in the deep red region of the visible spectrum. This wavelength can be efficiently transmitted through sclera and decreased the potential for photoreceptor and melanin absorption. This is of concern as transscleral laser light that exits the internal surface of the eye may have a potential for harming intraocular structures. In addition to photoreceptor and melanin interaction, exudative retinal detachment has been reported after direct retinal illumination with laser light for PDT. These detachments have been reported to occur after irradiation at 550 nm (120 mW/cm^2; 26.6 J/cm^2) [19] and 675 nm (120 mW/cm^2; 43 J/cm^2) [20]. These exudative detachments are thought secondary to transient increases in vascular permeability. Exudative retinal detachments were not observed in our study, and it is unlikely that irradiances and total doses from scattered light are comparable to conditions known to produce exudative retinal detachments.

Although the phthalocyanines are stable and have low systemic toxicity [9,20–22], there were some difficulties associated with the SINc used in the study. The most difficult problem is related to solubility. Because of low solubility, this compound was dissolved by heating and emulsified for delivery. The resultant large volume if delivered in comparable amounts to humans would require over a liter of hypotonic intravenous fluid. The potential risk to an elderly population of glaucoma patients may be unacceptable. Other methods of delivery may be possible and this photochemical may useful in topical applications. In order to make this compound more useful, both the absorption maxima and the solubility would need improvement. It may be possible to prepare derivatives of this compound that would increase solubility and shift absorption maxima into the commonly available diode ranges (790–830 nm). This shift into the near IR would maximize transscleral transmission, allowing lower irradiances and minimal interaction with melanin and photoreceptors.

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