Photodynamic Therapy for Corneal Neovascularization

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BACKGROUND AND OBJECTIVE: Photodynamic therapy with tin ethyl etiopurpurin (SnET2) was evaluated as a treatment modality for rat corneal neovascularization.

MATERIALS AND METHODS: Escalating light doses at 664 nm were applied focally to corneal neovascularization in rats 10 minutes following an intravenous injection of SnET2 using a low-power diode laser. Controls consisted of light-only and drug-only treatments. Clinical, angiographic, and histopathologic evaluations were performed on the animals up to 28 days after drug and/or light treatment.

RESULTS: A drug and light dose-response was seen in producing neovessel closure. In animals treated with SnET2 and the highest light dose (25 J/cm²), all eyes showed occlusion at every follow-up evaluation up to 28 days. Control eyes demonstrated progressive disease at all time points.

CONCLUSIONS: Photodynamic therapy appears to be safe and effective for eliciting prolonged (> 28 days) occlusion of corneal neovascularization in the rat model with minimal side effects and good clinical outcomes.

INTRODUCTION

Neovascularization plays a major role in many disease states within ophthalmology and oncology.¹,² Current treatments for the disease process have clinical limitations. Antiangiogenic steroidal drugs have minimal beneficial results with serious side effects, particularly with their long-term use.³ Thermal laser photocoagulation is overly destructive to adjacent normal tissues and has limited utility in the eye.⁴ Macular surgery and corneal transplantation are costly surgical procedures with risks requiring long-term follow-up care and often repeated surgery with questionable outcomes.⁵ Thus, new treatment strategies for neovascularization with minimal side effects are being sought.

PhotoPoint (Miravant Medical Technologies, Santa Barbara, CA), a form of photodynamic therapy, is an experimental therapeutic modality that holds tremendous potential in the treatment of neovascularization. The therapy involves the use of a light-activated pharmaceutical, localized in the target tissue, and a medical laser of an appropriate wavelength with continuous low-power light. Purlytin (tin ethyl etiopurpurin)
purin [SnET2], Miravant Pharmaceuticals, Inc., Santa Barbara, CA) is a light-activated pharmaceutical used in PhotoPoint that exhibits an affinity for hyperproliferating neovascular endothelial cells and other tissues. When Purlytin is exposed to 664-nm light, the excited drug transfers its energy to molecular oxygen, thus creating oxygen species that are harmful to cell function. PhotoPoint is a relatively selective modality that causes photosensitized oxidation of the proliferating cells while sparing the surrounding normal tissues. The experimental treatment is currently in multicentered clinical trials for the treatment of choroidal neovascularization secondary to age-related macular degeneration.

The objectives of this study were to assess an appropriate laser light dose for producing neovascular shutdown of corneal neovascularization in rats pretreated with intravenous Purltytin, and to evaluate the persistence of neovascular shutdown 1, 7, 14, and 28 days after PhotoPoint therapy.

**MATERIALS AND METHODS**

**Animal Model**

All animal experiments adhered to institutional guidelines and the ARVO Resolution on the Use of Animals in Research. Eighty-four male Sprague Dawley rats (Harlan Sprague Dawley, Indianapolis, IN), weighing 0.3 to 0.4 kg, were acclimated for 1 week prior to study enrollment. Prior to experimentation, the rats were anesthetized with an intramuscular injection of ketamine (100 mg/ml, Aveco Co., Inc., Fort Dodge, IA) mixed with acepromazine 1:10 and topical propracaine (0.5%) eyedrops. The pupil was dilated with 0.5% tropicamide. Under an ophthalmic surgical operating microscope (Applied Fiberoptics, Alcon, Fort Worth, TX), a suture (7-0 silk, Ethicon, Somerville, NJ) was passed into the corneal stroma, avoiding anterior chamber perforation. Subsequently, the suture was tied with a surgeon's knot plus an overlying square knot. Triple antibiotic ointment (neomycin, polymyxin b sulfate, and bacitracin) was applied to the eyes that were operated on.

Approximately 14 days later, the corneal neovascularization reached a treatable state and the suture was removed under anesthesia. One day after suture removal, baseline corneal fluorescein angiography was performed using sodium fluorescein (10% fluorescein, 0.75 mg/kg, Alliance Pharmaceuticals, Inc., Rich-mond, TX) administered via intravenous marginal tail vein injection. A minimum of five rats were used for each time point of evaluation. All experimental animals were compared with control animals in determining the efficacy of PhotoPoint treatment.

**Photosensitizer Administration**

Purltytin was supplied as a preformulated isotonic, isoosmotic lipid emulsion suitable for intravenous use at a concentration of 1.05 mg/ml (Lot KV1142B). Purltytin was injected intravenously as a slow bolus into the marginal tail vein (via a 24-gauge angiocatheter) at a dose of 1.0 mg/kg of body weight 1 day after baseline corneal fluorescein angiography. A drug dose of 1.0 mg/kg of Purlytin was chosen based on previous studies.

**Drug–Light Dose Schedule**

Laser light (664 ± 7 nm) at 1 of 3 different light doses (10, 15, or 20 J/cm² delivered at an irradiance of 150 mW/cm²) was applied focally to the cornea having neovascularization starting 10 minutes after Purltytin injection. The total laser power applied was 11 mW at the cornea, over a treatment light field of 3 mm in diameter. The light was delivered via a low-power diode laser (model DD3-0665, Miravant Systems, Inc., Santa Barbara, CA) with a slit-lamp adapter attached to a Haag-Streit slit-lamp biomicroscope (Haag-Streit 900, Bern, Switzerland). The vascularized cornea of each animal (n = 5 minimum per group) served as an untreated drug control. Light-only control rats (n = 10) were treated at the highest light dose (25 J/cm², 150 mW/cm²) without photosensitizer injection.

**Video Fluorescein Angiography**

Clinical and fluorescein angiographic examinations were documented via a slit-lamp video camera (Model LX-450, Optronics Engineering Corporation, Santa Barbara, CA) connected to a Sony (Shinegawa, Japan) VCR and monitor. The extent of neovascular shutdown was recorded and graphed. Tapes were stored for archival purposes. Representative 35-mm color photographic slides were made from the videos and stored on file.

**Histopathology**

After the animals were killed with a CO₂ overdose, the eyes were enucleated and fixed in 10% buffered formalin. Subsequently, histologic (hema-
toxylin-cosin staining) evaluations of corneal neovascular responses were made at various time points (1, 7, 14, and 28 days) after treatment.

**Data Analysis**

Data were collected, determined, and verified by an evaluator who was masked to the treatment procedures. Corneal neovascular occlusion was classified as complete, partial, no response, or progressive disease based on the fluorescein leakage following treatment. The post-treatment fluorescein angiograms were examined relative to the pretreatment baseline fluorescein angiograms. No evidence of fluorescein leakage over the area of light treatment was designated a complete response. The response of fluorescein leakage (< 50% of pretreatment angiogram) was termed a partial response. Eyes demonstrating more than 50% leakage were designated nonresponders. Progressive disease was noted by an increased fluorescein leakage compared with pretreatment levels. The occlusive results were summarized per group as the mean percent response at the corresponding time intervals following treatment. Microsoft Excel 4.0 (Microsoft Corporation, Seattle, WA) was used to perform descriptive statistical analysis. Vessel shutdown results were analyzed in a 3 × 4 × 2 table by logistic regression using JMP Statistics (SAS Institute, Inc., Cary, NC). The P value represents the odds of obtaining an objective response depending on light dose or time.

**RESULTS**

**Neovascular Induction**

Neovascularization was evident from 3 days post-suture and was expressed maximally 14 days after induction. Because inflammation was variable between fellow eyes, in most cases, the least inflamed eye was used as the control.

**Angiographic Responses to PhotoPoint**

Figure 1 demonstrates the percent response of eyes showing neovascular occlusion following PhotoPoint treatment. In animals treated with Purlytin and 10-J/cm² light, 5 of 6 eyes (83%) showed corneal neovascular shutdown at day 1, 5 of 5 eyes (100%) at day 7, 5 of 5 eyes (100%) at day 14, and 6 of 9 eyes (67%) at day 28. In animals treated with Purlytin and 15-J/cm² light, 7 of 7 eyes (100%) demonstrated neovascular shutdown at day 1 after light treatment, 5 of 5 eyes (100%) at day 7, 5 of 5 eyes (100%) at day 14, and 7 of 10 eyes (70%) at day 28. In the animals treated with Purlytin and 25-J/cm² light, 6 of 6 eyes (100%) showed corneal neovascular occlusion at day 1, 5 of 5 eyes (100%) at day 7, 5 of 5 eyes (100%) at day 14, and 6 of 6 eyes (100%) at day 28. Logistic regression modeling suggested that main effects of light (P = .63) and time (P = .72) were not significant. Therefore, the data provide no statistically significant evidence that the odds of an objective response (complete response and partial response) vary across light or...
time in this study. However, there appears to be a possible decrease in the odds of an objective response at day 28 for the 10 and 15 light-dose groups.

**Complete Response Rates**

The percent of eyes demonstrating complete responses is shown in Figure 2. In animals treated with Purlytin and 10-J/cm² light, 5 of 6 eyes (83%) showed complete corneal neovascular shutdown at day 1, 4 of 5 eyes (80%) at day 7, 3 of 5 eyes (60%) at day 14, and 3 of 9 eyes (33%) at day 28. In animals treated with Purlytin and 15-J/cm² light, 5 of 7 eyes (71%) demonstrated complete neovascular shutdown at day 1 after light treatment, 2 of 5 eyes (40%) at day 7, 2 of 5 eyes (40%) at day 14, and 1 of 10 eyes (10%) at day 28. In the animals treated with Purlytin and 25-J/cm² light, 6 of 6 eyes (100%) showed complete corneal neovascular occlusion at day 1, 3 of 5 eyes (60%) at day 7, 5 of 5 eyes (100%) at day 14, and 6 of 6 eyes (100%) at day 28.

**Clinical and Angiographic Examinations**

One to 2 days after PhotoPoint at the lowest light dose (10 J/cm²), the majority of eyes demonstrated no fluorescein staining in the area of light treatment (Fig. 3A) compared with pretreatment photographs (Fig. 3B). Persistent occlusion was seen at 1 and 2 weeks after PhotoPoint treatment with definite corneal clearing. Twenty-eight days after treatment, corneal surface irregularities diminished. Some treated vessels began to recanalize (Fig. 4). Neovascular endothelial cells contained pyknotic nuclei (Fig. 5).

At the second light dose tested (15 J/cm²), PhotoPoint caused vessel stasis over the central cornea with no fluorescein staining in the area of light treatment (Fig. 6). Ghost vessels were also present. Red blood cells appeared degenerated and crenated (Fig. 7). Twenty-eight days after treatment, there was marked clearing of the cornea.

At the highest light dose tested (25 J/cm²), PhotoPoint caused the greatest vessel stasis of neovessels in response to the light treatment (Fig. 8). Normal, underlying iris appeared unchanged. Ghost and atrophic vessels were also present. Light microscopy revealed vascular shutdown with fibrin and trapped red blood cells in the treated vessels. Corneal epithelium appeared normal. Minimal fluorescein staining was seen 14 (Fig. 9) and 28 days after
treatment, indicative of persistent vascular shutdown. Neovascular shutdown was noted exclusively in the cornea at all light doses tested. No evidence of toxicity was found in adjacent structures. The vascular supply in the iris, ciliary body, and retina was spared.

Control Responses

Fellow, drug-only control neovascularized corneas demonstrated progressive disease at all time points in the follow-up. None (0 of 10) of the eyes in animals for the light-only, no-drug control group exhibited vascular shutdown. Figure 10A represents an eye with corneal neovascularization that was treated with light only (25 J/cm²) 24 hours previously showing no clinical evidence of response to 664-nm light alone at the light parameters chosen. The corresponding fluorescein angiogram (Fig. 10B) reveals marked leakage over the light-treated field, confirming a lack of vaso-occlusive response to light only.

DISCUSSION

Current treatments for neovascularization have clinical limitations due to their side effects and minimal beneficial results. PhotoPoint, a treatment modality based on low-power light activation of a photosensitizer leading to localized, photochemical thrombosis, was evaluated in a rat model of corneal neovascularization. The objectives of the study were to evaluate the safety, effectiveness, and durability of the treatment with Purlytin.

Previously reported studies with the therapy have investigated the use of other photosensitizers in producing photothrombosis in experimental corneal neovascularization. This study used Purlytin with activation by a diode laser emitting 664-nm red light. The current study revealed that at low levels of 664-nm light, Purlytin caused vaso-occlusion of neovessels.
The two highest light doses tested (15 and 25 J/cm²) produced the greatest vaso-occlusive effect on neovascularized tissue. In addition, the persistence of vascular occlusion was dose related. The observation that opaque corneas, which were treated with PhotoPoint, became clearer may suggest that the neovascular endothelium is more sensitive to treatment than is the corneal endothelium. Vaso-occlusion was the result of damage to vascular endothelial cells leading to thrombus formation. This is consistent with the mechanism of action with other light-activated pharmaceuticals. Untreated fellow eyes and light-only control rats demonstrated continued fibrovascular proliferation and progression of corneal neovascularization. This demonstrates that both components, photosensitizer and light, are required for the therapy to produce the desired responses.

Histologic examination confirmed clinical observations. Adjacent ocular structures such as the iris, ciliary body, and retinal blood vessels did not demon-
strate vascular shutdown or damage. Neovascularization was occluded in the absence or presence of inflammation. The corneal epithelium remained intact in the area of laser irradiation and within the entire corneas. The stroma was initially edematous, but showed clearing throughout the study period. The corneal neovessels demonstrated stasis with red blood cell clumping. The nuclei of vascular endothelial cells were pyknotic. With the evolution of ghost vessels, the corneal stroma returned to normal. Descemet's membrane remained normal, as did the corneal endothelial layer. There was late clearing of the cornea at 28 days, indicating continued functioning despite neovascular shutdown.

PhotoPoint appears to be a safe and effective treatment for corneal neovascularization in the rat model. Minimal side effects and post-treatment complications were noted. The experimental therapy has the potential of being a noninvasive procedure with clinical outcomes superior to those of current therapies. Further investigation in other models and humans appears to be warranted.

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REFERENCES