Photodynamic Therapy of Intraocular Tumors Examination of Hematoporphyrin Derivative Distribution and Long-Term Damage in Rabbit Ocular Tissue

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ABSTRACT

Studies were performed to determine the distribution of hematoporphyrin derivative (HPD) in ocular structures and to characterize long-term damage associated with ocular HPD photodynamic therapy. Pigmented rabbits with an amelanotic melanoma heterotransplanted to the iris were used to obtain quantitative tissue levels of HPD as well as to document HPD localization by fluorescence microscopy. HPD was administered i.v., and tissue concentrations of HPD were determined by spectrofluorometry following porphyrin extraction. Vascular structures such as the tumor, iris, and choroid-retina as well as the aqueous fluid from eyes containing tumors demonstrated rapid HPD localization. The sclera had minimal HPD uptake, and the drug was not detected in avascular structures such as the lens or cornea. HPD was cleared from all ocular structures except the tumor and choroid-retina by 24 h following injection. Fluorescence microscopy data indicate that HPD remained in the vascular photoreceptor cell outer segments of the retina. Long-term damage was documented in rabbits which received HPD photodynamic therapy to a 1-sq cm area of retina via transpupillary light delivery. Acute damage to the exposed area of retina (in the form of a chorioretinal scar) could be induced. This damage was permanent but not progressive. Lens opacities were not observed, and the cornea, aqueous, and vitreous remained clear on all test eyes. The results from these studies suggest that HPD photodynamic therapy may provide a selective and safe approach to the treatment of ocular tumors.

INTRODUCTION

Clinical treatment of solid tumors by HPD PDT continues to show promise (1, 2). HPD exhibits properties of both preferential tumor localization and visible light-activated tumor destruction via photosensitization (3, 4). A general treatment protocol for PDT consists of i.v. administration of HPD followed 2 to 3 days later by local exposure of the tumor to visible light. Red light is most often utilized in PDT due to the superior tissue penetration characteristics of this wavelength (5), and the tumor destruction following HPD PDT is believed to be caused by singlet oxygen (\(^{1}\text{O}_2\)) which is produced via a type II photochemical reaction (4). Advances in the development of laser systems and procedures for light delivery, as well as continued preclinical research, have led to the utilization of HPD PDT for the treatment of numerous types of malignant tumors (6–14). Several current reviews describe the preclinical and clinical HPD investigations performed over the past 5 to 7 years (1, 2, 15, 16).

A recent application of HPD PDT is for the treatment of intraocular tumors such as uveal melanoma and retinoblastoma (7, 14, 17). The accessibility of ocular tumors and the optical properties of the eye appear to be compatible with HPD PDT. Depending on the clinical situation, most ocular tumors are currently treated by combinations of periodic observation, cryotherapy, photocoagulation, radioactive plaques, external beam radiation therapy, or enucleation (18–21). Unfortunately, each of these clinical modalities has various limitations or disadvantages which suggest that new types of therapy should be investigated. Cryotherapy and photocoagulation are effective only in the treatment of small lesions (21). Retinoblastoma is considered a radiation-sensitive tumor, but recent surveys report up to 50% failure rates following external beam radiation for unilateral and bilateral lesions (22). In addition, there is evidence that radiotherapy for bilateral retinoblastoma may play a role in the appearance of second primary tumors (22). Brachytherapy using \(^{60}\text{Co}\) plaques is effective in destroying choroidal melanoma, but failure rates of approximately 30% are reported (23), and intraocular complications frequently occur (24). Charged particles (protons and helium ions) offer improved dose distributions and possibly enhanced biological advantages for the treatment of choroidal melanomas (25, 26). However, the expense necessary to build, maintain, and operate cyclotrons suggests that widespread use of these modalities will be difficult to achieve. Since the size and placement of ocular tumors are often limiting factors in the effectiveness of current therapies, it is necessary to explore new methods of treating primary and recurrent tumors of the eye.

HPD-induced fluorescence is observed in transplanted anterior chamber tumors in the hamster and rabbit (27). In addition, porphyrin-induced fluorescence is observed in an amelanotic melanoma transplanted in the rabbit choroid following HPD administration (28). Human retinoblastoma heterotransplanted to nude mouse eyes retains \(^{3}\text{H}\)HPD, and this tumor model can be destroyed by HPD PDT (29, 30). Acute normal ocular tissue damage can be induced in rabbits by HPD PDT (30, 31). Damage in the form of retinal edema, detachment, and necrosis is seen within 2 days of ocular PDT, but the area of damage is limited to the treatment field in all but the highest doses of PDT. The use of HPD PDT in treating anterior chamber tumors in rabbits has been described by several groups (32–34). In most cases, a rapid alteration in the appearance of the tumor appears within 24 h of treatment and is characterized by Blanching, edema, and hemorrhage. Anterior chamber amelanotic melanomas measuring 4 to 5 mm in diameter and 2 to 3 mm high can be completely...
eradicated using a HPD PDT protocol consisting of HPD (5 mg/kg) and 90- to 180-J/sq cm dose of 630 nm light delivered at a power density of 150 milliwatts/sq cm (32). HPD PDT has been used clinically to treat select cases of uveal melanoma and retinoblastoma (7, 14). Most ocular tumors respond to PDT by exhibiting posttreatment hemorrhage and blanching, but long-term follow-up of these patients is not yet available. It is clear, however, that additional preclinical studies are needed prior to the initiation of meaningful clinical trials. The experiments described in this paper were designed to document HPD distribution in ocular structures and to characterize the long-term toxicity effects of ocular HPD PDT.

MATERIALS AND METHODS

Drugs. HPD was obtained from Photofrin Medical, Inc., Cheektowaga, NY, and was kept at 4°C in the dark until used. A solution of ketamine (30 mg/kg), acepromazine 93 mg/kg), and atropine sulfate (0.15 mg/kg) was administered i.m. to anesthetize animals prior to all experimental procedures. A solution of 1% cyclopentolate and 10% phenylephrine hydrochloride was used to dilate pupils and i.v. sodium pentobarbital (120 mg/kg) was used for euthanasia.

Animal and Tumor Models. Pigmented rabbits weighing approximately 2 kg were obtained from ABC Rabbitry, Pomona, CA, and were entered into studies 1 week after delivery. The amelanotic hamster melanoma heterotransplanted onto the iris of the rabbit eye was used in all HPD tissue distribution studies. Single-nodule tumors were obtained by placing a 1 mm³ piece of viable tumor (obtained from a stock tumor growing in the anterior chamber of a donor rabbit) onto the iris through a paracentesis incision at the limbus. The growth rate of the lesion is approximately 1 mm in diameter per day (31). Studies which involved tumors were initiated when the lesions were between 5 and 8 mm in diameter (approximately 10 days following transplant).

Light Source and Delivery Systems. All HPD PDT experiments were performed with a dye laser (using Krypton red dye) pumped by a 5-watt argon ion laser (Spectra Physics, Mountain View, CA). The output wavelength from the dye laser was set at 630 nm and checked with a scanning monochromator (Model 210; Coherent Radiation, Palo Alto, CA). Light from the laser was interfaced via a spatial filter (Spectra Physics) through a single-stop quartz fiber optic bundle. A microlens was fitted on the distal end of the fiber optic for production of a uniform light treatment field (34).

An 18-watt krypton ion laser running at 407 to 413 nm (Coherent Radiation) was used to visualize porphyrin-induced fluorescence in tissue precipitates.

HPD Distribution in Ocular Tissue. Rabbits with amelanotic melanoma growing on the iris of one eye received an i.v. injection of HPD at a dose of either 5 mg/kg for quantitative uptake studies or 10 mg/kg for fluorescence microscopy studies. At selected time intervals following injection, the animals were sacrificed, and ocular structures and fluids (cornea, aqueous, tumor, iris, lens, vitreous, retina-choroid, and sclera) were collected. The retina and choroid from each test eye had to be combined in order to obtain enough material for porphyrin determinations. Specimens were either assayed for quantitative porphyrin content using a tissue extraction procedure or examined by fluorescence microscopy. A micro lens was fitted on the distal end of the fiber optic for production of a uniform light treatment field (34).

An 18-watt krypton ion laser running at 407 to 413 nm (Coherent Radiation) was used to visualize porphyrin-induced fluorescence in tissue precipitates.

RESULTS

The distribution of HPD in ocular structures and fluids is shown in Table 1. Vascular structures such as the iris, choroid-retina, and tumor all demonstrated rapid localization of HPD. In addition, the aqueous in eyes with anterior chamber tumors also accumulated significant levels of HPD soon after drug administration. HPD was not detected in avascular structures such as the cornea or lens. The sclera and aqueous (from eyes without tumors) had extremely low concentrations of HPD at early time intervals after drug administration. At extended time periods following injection (24 h), HPD was cleared from the iris, sclera, and aqueous. HPD levels remained high in the tumor and choroid-retina at both 24 and 48 h.

Phase-contrast and fluorescence micrographs of ocular structures were obtained at various time intervals following HPD administration. Micrographs of tumor tissue at 6 h following drug injection are shown in Fig. 1. While HPD was present in the entire tumor, the highest intensity of fluorescence was observed...
in what appeared to be necrotic areas of the tumor mass (Fig. 1, arrow). HPD was not observed to localize in or around clearly identifiable vascular channels (Fig. 1, open arrow). Fluorescence localization patterns of HPD in ocular structures are shown in Figs. 2 to 4. Figs. 2 and 3 illustrate the HPD distribution in the choroid body (Fig. 2) and the choroid-retina (Fig. 3) at 30 min following injection. At this time point, HPD fluorescence was localized primarily to the small cells or capillaries in the ciliary processes (Fig. 2, small arrows) and to the ciliary epithelium (Fig. 2, arrowhead). In addition, HPD was observed in the choroid and retinal pigment epithelium (Fig. 3). Minimal fluorescence was seen in the photoreceptor cell outer segments of the retina at this time period, and fluorescence was not observed in the sclera. Fig. 4 shows the HPD distribution in the choroid-retina at 24 h following drug injection. At this time period, HPD fluorescence was observed in the retinal epithelium and the retinal photoreceptor cell outer segments.

Table 2 summarizes the effects of HPD PDT which relate to long-term ocular damage. The length of follow-up ranged from 9 to 30 months. Damage to the exposed area of retina was observed and documented using the techniques of fundus photography, fluorescein angiography, and histopathological examination. The retinal damage induced by HPD PDT was a chorioretinal scar. Slit-lamp examinations demonstrated normal suture lines in the lenses of all test eyes, but lens opacities were not observed. In addition, the cornea, aqueous, and vitreous of all test eyes remained clear throughout the follow-up period.

Fig. 5 illustrates typical fundus photographs of Rabbit Eye 5 from the long-term chronic damage study. Fig. 5A shows the rabbit retina prior to treatment. The area of acute choriotreinal damage observed 2 days following HPD PDT (10 mg/kg, 90 J/sq cm) is shown in Fig. 5B. The damaged area appeared similar to that observed previously in acute toxicity experiments (30), and this would suggest that retinal detachment as well as necrosis was induced. Fig. 5C and D documents the appearance of the treatment area at 7 days and 6 months, respectively. The chorioretinal scar was present 21 months following treatment as shown in Fig. 5E. Retina outside the treatment field appeared normal as determined by fundus photography, fluorescein angiography, and histopathological examination.

**DISCUSSION**

Localization of HPD in ocular structures and characterization of long-term ocular toxicity following HPD PDT have been obtained using a rabbit model. Current experiments were designed in order to document the types and severity of ocular toxicity which could be induced by HPD PDT under extreme parameters. The amelanotic melanoma can be heterotransplanted to either the choroid or iris of rabbits. While the choroidal placement is a more realistic anatomical location (most human ocular tumors are choroidal), we utilized experimental iris transplants to either the choroid or iris of rabbits. While the choroidal placement is a more realistic anatomical location (most human ocular tumors are choroidal), we utilized experimental iris
tumors to ensure that a standard tumor size was examined in all HPD uptake studies.

The pharmacokinetics of HPD has been examined previously using radiolabeled compounds (\[^{14}C\] and \[^{3}H\]HPD) or by spectrofluorometric techniques. The use of \[^{14}C\]HPD in large animals is cost prohibitive, and \[^{3}H\]HPD can degrade to produce radioactive contaminants (37). Therefore, the uptake and distribution of HPD in ocular structures were documented using a spectrofluorometric procedure following porphyrin extraction from tissue. The efficiency of any drug extraction techniques may be a potential variable. In our experiments, however, the tissue precipitates which were obtained following extraction did not contain detectable levels of porphyrin fluorescence. While this indicates that a high percentage of HPD was extracted from the tissue specimens, it does not rule out the possibility that HPD-induced fluorescence may be quenched by pigmented tissue precipitates of iris or choroid. Fluorescence microscopy data correlated with the quantitative spectrofluorometric data with respect to documenting which ocular structures accumulated HPD. Results from our study indicate that HPD was retained in an experimental ocular melanoma. These findings confirm an earlier investigation describing HPD-induced fluorescence in the same tumor when it is transplanted to the choroid of the rabbit eye (28). The high levels of HPD in the aqueous of tumor-containing eyes indicated that porphyrin leaks from incompetent vessels of the tumor and that alterations to the blood-aqueous barrier may be induced. The concentrations of HPD found in the experimental ocular tumor may be an underestimate of the potential clinical situation due to the rapid growth rate of this cancer. Previous experiments indicate that the amelanotic melanoma increases in diameter by 1 mm/day when grown on the iris (32). It would be interesting to determine HPD concentrations in a tumor with a slower (and therefore clinically relevant) growth rate. Fluorescence microscopy of malignant tissue showed that the highest amount of HPD (at all time points examined) was in what appeared to be necrotic areas of the tumor. The presence of phagocytic macrophages in necrotic areas of the tumor may play a role in the observed high level of HPD fluorescence in this area. However, histopathological examination of PDT-treated tumors show rapid and severe vascular damage, which suggests that significant levels of HPD are present in these well-oxygenated areas of tumors (1, 32).

Our study also demonstrated that the iris and choroid-retina accumulate significant levels of HPD while the sclera retained low amounts of porphyrin. HPD was not detected in avascular structures such as the cornea and lens. HPD was rapidly taken up in the iris and was also cleared from this structure. The pharmacokinetics of HPD in the choroid-retina shows both a rapid uptake and a partial clearance following systemic injection. However, HPD levels in the choroid-retina remained high even out to 48 h. Fluorescence microscopy data indicate that HPD concentrates first in the choroid microvasculature, is taken up by the retinal pigment epithelium, and then localizes within the outer segments of the retina. The retina of the rabbit eye is not vascularized (except for the medullary ray), and the filtering properties of the vitreous are poor. Therefore, HPD may not be easily cleared from the retina. Instead, HPD remains within the neurosensory outer segments at concentrations equal to or higher than that observed in tumor tissue. The effects of a vascularized retina, as found in humans, on HPD localization is not known and precludes any accurate comparison of HPD distribution in the retina of rabbits and humans.

The low concentration of HPD found in the sclera suggests that transscleral light delivery may be an effective and safe method of treating the base of ocular melanomas. Most choroidal and ciliary body melanomas are pigmented and thicker than 5 mm (18). A PDT treatment which combines transpupillary and transscleral light delivery might provide a more effective procedure for complete tumor eradication. Additional studies are needed in this area, but it would appear from our HPD distribution experiments that photodynamic injury to the sclera would be minimal.

The absence of HPD in the cornea and lens correlates with the lack of observable acute or chronic damage to these structures following HPD PDT. An earlier study demonstrated that acute ocular damage in the form of well-circumscribed areas of retinal edema, detachment, and necrosis could be induced by HPD PDT (31). Our current study, which had a follow-up period which ranged from 9 to 30 months, demonstrated that the retinal damage was localized and not progressive. The ability to accurately localize the beam of light used in treating ocular tumors should minimize the amount of surrounding retina which is exposed and therefore the amount of normal retina at risk for permanent damage. The facts that HPD was not detected in the lens of any experimental eyes and that lens opacities were not observed during the follow-up examination period suggest that HPD PDT will not produce complications such as cataracts. In agreement with these observations is the fact that we have observed patients treated with ocular HPD PDT for up to 24 months without evidence of any corneal or lens opacities. However, photosensitizers such as chlorpromazine, promazine, and hematoporphyrin can induce in vitro photopolymerization and histidine destruction in calf lens protein (38). In addition, 8-methoxypsoralen (which is used in phototherapy of psoriasis) can induce cataracts (39). Therefore, potential problems related to lens damage induced by HPD PDT should not be ruled out.

REFERENCES


4 A. L. Murphree, unpublished data.


Fig. 5. Clinical appearance of Rabbit Eye 5 prior to treatment and at various time intervals following PDT (10 mg HPD/kg, 90 J/sq cm). A, fundus photograph taken prior to treatment. B, fundus photograph taken 2 days posttreatment. Retinal detachment and folds are seen within the treatment field. C, fundus photograph taken 7 days posttreatment. D, 6 months posttreatment. E, 21 months posttreatment. Retinal vasculature within the medullary ray appears unchanged from pretreatment observations. The area of retinal damage has remained constant throughout the observation period.
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