Hematoporphyrin Derivative Photofacitation Therapy for the Treatment of Intraocular Tumors: Examination of Acute Normal Ocular Tissue Toxicity

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ABSTRACT

Preclinical studies designed to define potential side effects resulting from the use of hematoporphyrin derivative (HPD) photofacitation therapy (PRT) as a modality for treating intraocular tumors have been performed. Pigmented rabbits were used to evaluate acute normal ocular tissue toxicity following single HPD PRT treatments in which the light was directed through the pupil and onto a 1-sq cm area of the retina. The treatment procedure consisted of the i.v. administration of HPD (1 to 10 mg/kg) followed 48 hr later by a 15-min exposure of localized red light [635± 5 nm; 40 to 400 milliwatts/sq cm] generated by a free running rhodamine B dye laser pumped by a 5-watt argon laser. Toxicity to normal retinal tissue was documented using fundus photography, fluorescein angiography, and histological examination. The results of this study demonstrated that ocular damage in the form of retinal edema, detachment, and necrosis could be induced by clinically relevant doses of HPD PRT. The area of retinal damage was limited to the treatment field in all but the highest doses of HPD PRT. The histological results were in agreement with the visual observations in that abrupt and demarcated transition areas between injured and normal-appearing retina were observed. Care will have to be used in the delivery of light to the treatment field if HPD PRT is to be utilized for treatment of intraocular tumors.

INTRODUCTION

There are approximately 1800 new cases of primary malignant tumors of the eye diagnosed each year in the United States (3). In addition, there are approximately 400 deaths per year resulting from the consequences of this type of disease (3). Malignant melanoma of the choroid comprises about 75% of all primary ocular tumors while retinoblastoma accounts for about 20% of these tumors. Radiation therapy is often the primary treatment modality for intraocular tumors in patients with useful vision. External beam irradiation is beneficial in the treatment of retinoblastoma, and brachytherapy using ²¹⁰Po plaques is effective in destroying choroidal melanoma (1, 3, 23, 25, 26). Unfortunately, the overall success of radiation therapy for intraocular cancer is not totally satisfactory. Ocular complications such as radiation retinopathy and local tumor recurrence are frequently observed following radiation therapy (3, 5, 18, 25). A recent survey of 50 cases of unilateral retinoblastoma treated with external beam irradiation reported a 50% failure rate requiring additional treatment (1). The failure was due either to the development of new tumors or to the lack of complete tumor response. There is also a 50% failure rate in 37 cases of bilateral retinoblastoma in which both eyes are treated simultaneously with radiation (1). An analysis of 107 cases of choroidal melanoma treated with ²¹⁰Po plaques reports 12 deaths related to the melanoma and 27 eyes which required enucleation due to failure or complications of treatment (23). Modalities such as photocoagulation and cryotherapy are used as alternative or adjunctive treatment for intraocular tumors, but these therapies are useful only against small lesions (2, 25, 27). It is apparent that additional methods of treating primary as well as recurrent intraocular tumors are needed.

The anatomical and physiological properties of the eye appear to be compatible with HPD PRT, and this modality of treatment may be beneficial for the improved management of intraocular tumors. The selectivity with which tumors are destroyed following HPD PRT as well as the ability to use HPD PRT in areas treated previously with ionizing radiation are characteristics of this therapy which could be exploited in patients with intraocular tumors (10). The basic methodology involved in the use of HPD PRT consists of an i.v. injection of HPD, which is followed 2 to 5 days later by a localized exposure of the malignant lesion to visible red light of wavelengths between 625 and 640 nm. The preferential accumulation of HPD in malignant tissue compared to adjacent normal tissue (14, 22) and the photodynamically induced generation of cytotoxic singlet oxygen by HPD when illuminated with visible light (28) account for the effectiveness of this treatment. Currently, HPD PRT is under clinical investigation as a new modality to treat a wide variety of solid tumors (7, 8, 10, 11, 19, 21, 24).

Information pertaining to the localization and/or the phototherapeutic potential of HPD as it relates to ocular tumors is limited. HPD fluorescence is observed in melanomas transplanted to the anterior chamber in hamsters and in carcinomas transplanted to the anterior chamber in rabbits (6). Increased fluorescence of HPD (tumor versus normal ocular tissue) is also observed following i.v. injections of the drug in rabbits with an amelanotic melanoma growing in the choroid (20). Eyes of athymic mice containing human retinoblastoma accumulate higher concentrations of ³H-HPD than control eyes (15), and this tumor can be destroyed by HPD PRT (2). A preliminary study demonstrated that both macroscopic and microscopic...
retinal damage are induced in rabbits following daily injections of HPD (37 mg/kg) and daily 4-hr exposures of white light (12). The damage resulting from these high levels of HPD and light included pigment changes and extensive degeneration of the retina.

It will be necessary to examine potential ocular complications resulting from HPD PRT prior to initiating extensive clinical studies using this therapy to treat tumors of the eye. In this paper, we present the results from preclinical studies which were designed to document both the type and extent of acute normal ocular tissue toxicity induced in pigmented rabbits following HPD PRT.

MATERIALS AND METHODS

Drugs. HPD was obtained from Oncology Research and Development, Inc., Cheeketowaga, N.Y. It was supplied as a sterile solution at a concentration of 5 mg/ml in 0.9% NaCl solution. An i.m. injection of a combination of ketamine (30 mg/kg), acepromazine (3 mg/kg), and atropine sulfate (0.15 mg/kg) was used to anesthetize animals prior to all experimental procedures. Animals were sacrificed using an i.v. overdose of sodium pentobarbital (120 mg/kg). The pupils of experimental animals were dilated using a solution consisting of 1% cyclopentolate hydrochloride and 10% phenylephrine hydrochloride.

Animals. Eight- to 12-week-old pigmented rabbits were obtained from ABC Rabbitry, Pomona, Calif., and were entered into experiments 1 week after delivery. The animals were maintained on a normal diet consisting of rabbit chow and water ad libitum.

Light Source and Delivery Systems. Red light at 635 nm ± 5 nm was generated by a free-running rhodamine B dye laser pumped by a 5-watt argon laser (Spectra-Physics Inc., Mountain View, Calif.). During photoradiation treatments, the light was delivered through a 200-μm silica fiber. The intensity of light was measured with a thermopile (Model 210; Coherent Radiation, Palo Alto, Calif.). The wavelength of light emitted from the dye laser was measured with a scanning monochromator (American ISA, Inc., Metuchen, N.J.).

Acute Normal Ocular Tissue Toxicity. Normal pigmented rabbits were administered a single i.v. injection of HPD (1, 2.5, 5, or 10 mg/kg), and then 48 hr later a 1-sq cm area of the fundus of each test eye received a 15-min exposure to light at 635 ± 5 nm. The light was delivered from the tip of a 200-μm-diameter silica fiber, which was modified to provide light delivery with a full angle divergence of 46°. The fiber was positioned in the center of the dilated pupil and was placed 2 mm from the surface of the cornea. Measurements of the transmitted light pattern on the posterior surface of the sclera were made on an enucleated eye and confirmed that the light delivery procedure produced a 1-sq cm treatment field on the rabbit fundus. The eyelids were retracted with a lid speculum prior to treatment, and the cornea was moistened with 0.9% NaCl solution during the light exposure. Due to the positioning of the silica fiber, the treatment field was situated on or near the optic disc and medullary ray. The irradiance of the red light on the exposed retina was calculated by assuming 93% transmission of 635-nm light (13) and was either 40 or 100 milliwatts/sq cm for animals given injections of HPD or 100, 200, 300, or 400 milliwatts/sq cm for animals which did not receive the drug. The percentage of 635-nm light absorbed by the retina does not exceed 2% whereas the percentage of 635-nm light absorbed by the retinal pigment epithelium and choroid is approximately 57% (13). A total of 45 eyes from 35 rabbits were treated in this manner and then analyzed for acute normal ocular tissue toxicity using fundus photography, fluorescein angiography, and histopathological examination.

Visualization of the rabbit fundus and photographic documentation of the HPD PRT treatment was obtained using an Equator Plus Camera equipped with direct illumination and a 148° wide angle lens (Medical Instruments Research Associates, Waltham, Mass.). The animals were anesthetized, and the pupils were dilated prior to photography. Fundus photographs were taken prior to treatment, immediately after treatment, and 2, 7, and 14 days after treatment.

Fluorescein angiograms were performed on treated eyes either on Day 10 or Day 11 following HPD PRT. The pupil of the test eye was dilated, and the animal was anesthetized prior to the fluorescein angiography procedure. The cornea was placed in contact with the lens of the Equator Plus Camera, and then 0.5 ml of a 10% solution of sodium fluorescein (Alcon Laboratories, Fort Worth, Texas) was injected into the marginal ear vein. Serial photographs were then obtained using 460- to 480-nm light to excite the fluorescein. A 520-nm barrier filter was used to record the fluorescence of the dye. Pictures were taken at a rate of 1 frame/sec for the first 30 sec following injection of the fluorescein, and then single frames were taken at 1, 2, 5, 10, and 20 min after injection.

The rabbits were sacrificed 14 days after HPD PRT. The test eyes of each rabbit were enucleated and fixed in half-strength Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M sodium phosphate buffer). Specimens from the region of treatment (including the optic disc, medullary ray, and exposed areas of the retina) as well as areas adjacent to the treatment field were then dehydrated in a routine stepwise procedure using ethanol. After dehydration, the specimens were embedded in glycol-methacrylate and sectioned on a Sorvall microtome. The sections were then stained with hematoxylin and eosin.

RESULTS

Acute toxicity to normal ocular tissue induced by HPD PRT was assessed by documentation of fundus appearance, fluorescein angiography, and histological examination. Table 1 summarizes the effects of HPD PRT in terms of acute normal tissue toxicity for all treated eyes. No toxicity was observed either visually or at the light microscopic level by treatments of HPD alone, HPD plus room light, or localized 635 ± 5 nm light exposure if the light intensity was kept below 200 milliwatts/sq cm. A 15-min exposure of 635 ± 5 nm light at intensities of 300 or 400 milliwatts/sq cm (in the absence of HPD) induced a demarcated opaque retinal lesion. Ocular side effects such as corneal or lens damage were not observed either visually or histologically in any of the eyes treated with HPD PRT.

Examples of the type of information obtained from a control eye (Eye 3) are shown in Fig. 1. A fundus photograph illustrating the normal visual appearance of the posterior segment of the rabbit retina is shown in Fig. 1A. A normal fluorescein angiogram is shown in Fig. 1B. The angiogram utilizes the fluorescent properties of sodium fluorescein to examine the anatomical and physiological integrity of the retina and choroidal vasculature. This angiogram illustrates the normal retinal circulation of the dye in the arterial and venous vessels located in the medullary ray. The background fluorescence is typical of vascular filling in the choroid. The histological appearance of the posterior segment of Eye 3 is shown in Fig. 1C. The retina is composed of photoreceptor cells (rods and cones), bipolar and horizontal cells, and ganglion cells. The retina is separated from the highly vascularized choroid and choriocapillaris by the retinal pigment epithelium and Bruch's membrane.

An example of minimal ocular toxicity observed following HPD PRT is shown in Fig. 2. A fundus photograph taken 7 days following treatment (5 mg HPD per kg; 40 milliwatts per sq cm; Eye 29) is shown in Fig. 2A. The delivered light dose of 36 J/sq cm was in the range of a medium dose used clinically for the treatment of superficial skin tumors (8). In addition, the concentration of HPD administered to this rabbit (5 mg/kg) was at the upper end of that used clinically. The treatment field...
was confined to the posterior segment of the retina inferior to the medullary ray. A well-defined area of retinal hypopigmentation was observed in the fundus. There was no evidence of retinal detachment in the treatment field, and the fundus outside the treatment field appeared normal. The area of retinal disturbance was still visible 14 days after treatment. Fig. 2B is the corresponding fluorescein angiogram. A normal-appearing medullary ray with typical vascular filling was seen. A patch of granular hyperfluorescence was present in the area in which retinal hypopigmentation had been identified. The remainder of the fundus appeared normal. Fig. 2C is a light micrograph of a section through the damaged region of the retina. The histopathological findings include minor loss of some outer photoreceptor segments along with migration of cells and leakage of fluid into subretinal space. The majority of the retina appeared normal, as did the choroid.

Fig. 3 illustrates the observations obtained following HPD PRT treatment, which consisted of 5 mg HPD per kg and 100 milliwatts per sq cm (Eye 31). The appearance of the fundus 7 days following treatment is shown in Fig. 3A. A circumscribed and opaque area of detached retina was present over the entire treatment field. No hemorrhage was observed in the fundus, and both the optic disc and medullary rays appeared normal. By Day 14, the retina had settled down but remained opaque and atrophic in appearance. The results of a fluorescein angiography performed on this eye are shown in Fig. 3B. The retinal vessels located in the medullary ray were not in the treatment field and therefore appear normal. The treatment field was defined by a demarcated area of hypofluorescence which corresponds to the damaged area observed with fundus photography. A light micrograph illustrating the histopathological findings is shown in Fig. 3C. Full-thickness necrosis of the retina together with a firm adhesion between residual retina and choroid was observed. The destruction of the photoreceptor cells and the collapse of the outer plexiform layer resulted in a poorly defined single nuclear layer. An abrupt fold separated the damaged area from architecturally intact retina. Examples of toxicity induced by the highest dosage of HPD PRT used in this study were similar to that illustrated in Fig. 3 except for an increase in the area of damage. Fig. 4 illustrates the typical fundus and angiogram observations following a HPD PRT treatment with 10 mg HPD per kg and 100 milliwatts per sq cm (Eye 38). Fig. 4A is a fundus photograph taken 2 days posttreatment and shows that massive retinal detachment (with retinal folds) was induced. Hemorrhage was present on the retina and extended outside the treatment field. By the end of the 14-day observation period, the hemorrhage had dissipated, but the area of atrophic and opaque retina remained larger than the treatment field. Fig. 4B illustrates the results of a fluorescein angiogram. The area of hypofluorescence corresponded to the damaged area observed with fundus photography. In addition, since the treatment field included portions of the medullary ray, there was retinal vessel occlusion on both sides of the optic nerve. The histological findings were similar to those observed in Fig. 3C in that there was complete retinal necrosis in the treatment field.

DISCUSSION

The effectiveness of HPD PRT in destroying malignant tumor tissue is well documented (2, 7–9, 11, 19), and there is relatively little reported about complications arising from this therapy other than an occasional transient skin photosensitization reaction (10, 11). However, HPD does accumulate in normal tissue (14, 15) and, therefore, photodynamically induced damage to normal tissue must be considered a potential side effect of this therapy. The desire to expand the clinical application of HPD PRT to include the treatment of intracocular tumors necessitated the present examination of HPD PRT-induced normal ocular tissue toxicity.

The results obtained from this preclinical study demonstrated that normal retinal damage can be induced by HPD PRT. It is important to note that the range of HPD doses and the total
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powers of 635 ± 5 nm light utilized in this study are comparable to levels which are currently used clinically for other tumor types (10). As detailed in Table 1, a wide range of acute retinal responses (from mild retinal edema to complete retinal necrosis) were induced by HPD PRT. The severity of the damage correlated well with the dose of HPD PRT which was delivered. Ocular toxicity which includes damage to the retinal pigment epithelium and loss of photoreceptor layers can be considered irreversible and therefore unacceptable if produced to a significant extent outside the treatment field. Fortunately, the area of retinal damage was limited to the treatment field in all but the highest doses of HPD PRT. The histological results were in agreement with the visual observations in that well-demarcated transition areas between injured and normal-appearing retina were observed. In addition to retinal injury, both localized hemorrhage of retinal vessels and damage to the optic disc were recorded when high dose HPD PRT treatment included these areas of the retina. No damage to either the cornea or lens was observed following HPD PRT. The toxicity data indicate that extreme care in delivery of light to a malignant lesion in the eye will be needed in order to minimize potential damage to normal areas of the retina. Choroidal tissue was not as severely damaged as retinal tissue, and this suggests that transmission of red light to the choroid was inhibited by the overlying retinal pigment epithelium. Therefore, effective treatment of choroidal tumors may possibly require the use of transsceral illumination.

The observation that distinct retinal lesions could be induced with red light exposures at intensities greater than 200 milliwatts/sq cm in the absence of HPD indicates that thermal and/or direct photochemical interactions occurred. The lesion induced by the red light exposure was actually smaller than the treatment field, which suggests that thermal damage was mainly involved (17).

Management of intraocular tumors with current treatment modalities is often unsatisfactory, and enucleation is eventually necessary in many patients (3, 25). Experimental radiation therapies of intraocular melanomas using helium ions (4) or protons (16) are options which offer improved dose distribution and possibly enhanced biological advantages (for the densely ionizing helium ion). Unfortunately, the enormous expense necessary to build, maintain, and operate cyclotrons suggests that widespread use and acceptance of these modalities may be difficult to achieve. In addition, a recent case report described the failure of proton beam irradiation to effectively treat a malignant ciliary body melanoma (29). There are several properties of HPD PRT, in addition to the availability and relative simplicity of this treatment which indicate that this modality may be beneficial for the treatment of intraocular tumors. The preferential destruction of tumor tissue demonstrated clinically (7, 8) would be extremely beneficial in minimizing normal tissue complications when treating tumors of the eye. This treatment may prove to be useful for the management of recurrent intraocular tumors since the prior use of conventional radiation therapy does not preclude the subsequent use of HPD PRT (10). The usefulness of HPD PRT for treating pigmented ocular melanomas would appear to be promising since pigmented cutaneous melanomas are effectively radiated by HPD PRT in humans (7) as well as pet dogs (9).

In conclusion, the results of the acute normal toxicity study suggest that well-defined circumscribed areas of damage can be induced in eyes treated with HPD PRT. The lack of early complications to the anterior segment of the eye (cornea or lens) as well as to nonexposed areas of the retina is an indication that this modality may provide a relatively nontoxic method to effectively treat intraocular tumors. Examination of late effects of HPD PRT to normal ocular tissue will have to be completed prior to detailed evaluation of potential side effects of this treatment modality.

ACKNOWLEDGMENTS

The assistance of Robert Zink with coordinating the histological preparations and of Corey Mark with animal treatment procedures is greatly appreciated. We thank Albert L. Castorena for assistance in the preparation of this manuscript.

REFERENCES


Fig. 1. Visual and histological documentation of a normal rabbit retina (Eye 3). A, fundus photograph showing the posterior portion of the retina with the medullary nerve fiber radiating out from the optic disc. B, fluorescein angiogram illustrating normal retinal vessel filling of fluorescein and background fluorescence due to the choroidal vasculature. C, light micrograph of the retina-choroid showing the ganglion cell layer (G), inner nuclear layer (I), outer nuclear layer (O), and photoreceptor layer (P). The retinal pigment epithelium and choroid (C) are beneath the retina. H & E, × 400.
Fig. 2. Visual and histological documentation of Eye 29. 

A, fundus photograph taken 7 days posttreatment. A focal area of retinal hypopigmentation is inferior to the medullary ray. 

B, fluorescein angiogram illustrating focal area of hyperfluorescence. 

C, light micrograph of treated retina-choroid. The photoreceptor layer is damaged and the retinal pigment epithelium is disturbed. H & E, × 400.
Fig. 3. Visual and histological documentation of Eye 31. A, fundus photograph taken 7 days posttreatment. A circumscribed area of retinal detachment with retinal folds is inferior to the medullary ray. B, fluorescein angiogram illustrating circumscribed area of hypofluorescence. The retinal vessels appear normal. C, light micrograph of treated retina-choroid. There is an abrupt junction between normal and damaged tissue. Complete retina necrosis and a choroid-retinal adhesion are present in the treatment field. H & E, x 160.

Fig. 4. Visual documentation of Eye 38. A, fundus photograph taken 2 days posttreatment. Retinal detachment extends down to the optic disc. Hemorrhage is present in the medullary ray. B, fluorescein angiogram illustrating hypofluorescence extending outside of the treatment field. There is vessel occlusion in the medullary ray.
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