Development of a Novel Indwelling Balloon Applicator for Optimizing Light Delivery in Photodynamic Therapy

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INTRODUCTION

Although glioblastoma multiforme is often thought to be a disseminated tumor in the brain, failure of treatment is usually due to local recurrence at the site of surgical resection—in 80% of all cases, recurrence is within 2 cm of the resected margin [1]. This would indicate that a more aggressive local therapy could be of benefit.

Photodynamic therapy (PDT) is a local form of treatment involving the administration of a tumor-localizing photosensitizing drug that is activated by light of a specific wavelength [2]. This therapy results in a series of photochemical and photobiological events that cause irreversible photodamage to tumor tissues. PDT has several features that make it an effective adjuvant therapy in the treatment of brain tumors: (1) it is a local form of treatment in which the treated volume is limited by high attenuation of light in brain tissues; (2) resistance to PDT has not been encountered during treatments of brain tumors [3]; and (3) repeated applications of PDT is an option due to low long-term morbidity. The aim of PDT is to eliminate the nests of tumor cells remaining in the margins of the resection cavity; however, it is unlikely that standard “one-shot” intraoperative PDT treatments can accomplish this goal. This is primarily due to the limited penetration of light in brain tissues and the resulting long treatment times required to deliver sufficient light doses to depths of 1–2 cm in the resection cavity. A number of in vitro [4,5] and in vivo [6–9] studies suggest that the response to PDT depends not only on the total fluence, but also on the rate at which the fluence is delivered—lower fluence rates (i.e., longer treatment times) appear more efficacious in many instances. In addition, the results of preliminary clinical trials suggest that patient outcome is correlated to fluence—higher fluences result in longer survival [10]. The combination of high fluences and low fluence rates imply treatment times of the order of hours. Since the efficacy of PDT depends on the ability to deliver adequate fluences to malignant cells in the resection margin, careful consideration must be given to the light delivery technique. Short-term intraoperative light delivery is unlikely to eradicate tumor cells deep in the margin due to the inability to deliver threshold fluences in a

Background and Objective: A human glioma spheroid model is used to investigate the efficacy of different light delivery schemes in 5-aminolevulinic acid (ALA)—mediated photodynamic therapy (PDT). The results provide the rationale for the development of an indwelling balloon applicator for optimizing light delivery.

Study Design/Materials and Methods: Human glioma spheroids were incubated in ALA (100 or 1000 µg ml⁻¹) for 4 hours and subjected to various light irradiation schemes. In one set of experiments, spheroid survival was monitored as a function of light fluence rate (5–200 mW cm⁻²). In all cases, spheroids were exposed to fluences of either 25 or 50 J cm⁻². In a second study, the effects of repeated weekly PDT treatments, using sub-threshold fluences, were investigated. One group of spheroids was subjected to three treatments using fluences of 12, 12, and 25 J cm⁻². Results were compared to spheroids receiving single treatments of either 12 or 25 J cm⁻². A fluence rate of 25 mW cm⁻² was used for all three groups of spheroids. In all cases, the effect of a given irradiation scheme was evaluated by monitoring spheroid growth.

Results: Low fluence rates produce greater cell kill than high fluence rates. The minimum effective fluence rate in human glioma spheroids is approximately 10 mW cm⁻². Repeated weekly PDT treatments with sub-threshold fluences result in significant cell kill. In spheroids surviving the PDT treatments, growth is suppressed for the duration of the treatment period.


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Key words: ALA; fluence rate; fractionated PDT; glioblastoma multiforme; glioma spheroids

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reasonable time period. A solution to the problem of inadequate light delivery is to insert an applicator into the resection cavity thus allowing light delivery over extended time periods. Such an applicator would also facilitate investigation of other light delivery schemes, such as fractionation and long-term repeated PDT treatments. Unfortunately, currently used photosensitizers are ill suited to such treatment regimens.

Porphyrians, such as hematoporphyrin derivative and Photofrin \( ^{\text{R}} \), have been used almost exclusively in clinical PDT trials of the brain. Although favorable results have been reported by a number of investigators \([11,12]\), these photosensitizers are not suitable for use in fractionated PDT treatment regimens due to their accumulation in cutaneous tissues. The uncommonly long period of cutaneous photosensitization (lasting up to several weeks) negatively impacts the patient’s quality-of-life. Due to the drawbacks of traditional porphyrins, other photosensitizers, such as 5-aminolevulinic acid (ALA) are currently being evaluated for use in PDT of gliomas \([3,13]\).

ALA has been used primarily as a topical agent in the treatment of superficial skin lesions \([14]\); however, the abundance of ALA-induced Protoporphyrin IX (Pp IX) in rapidly proliferating cells of many tissues provides a biologic rationale for ALA-mediated PDT in a wide variety of lesions \([15]\). The combination of excellent tumor-to-normal brain tissue localization \([16]\), short period of skin photosensitization (24–48 hours) and the possibility of oral administration, makes ALA an ideal photosensitizer for use in fractionated or repeated PDT treatments of glioma patients.

In this study, the response of human glioma spheroids to ALA-mediated PDT using various light delivery regimens is investigated. Of particular interest are the effects of long term repeated PDT treatments on spheroid survival. Effects of fluence and fluence rate are also investigated. The results suggest that repeated PDT given over several weeks is more effective than single treatments. In addition, the results confirm the findings of other studies, namely that fluences are more effective if given at low fluence rates. Taken together, these results provide the rationale for the development of an indwelling balloon applicator that will allow chronic PDT treatments in patients over extended time periods.

MATERIALS AND METHODS

Cell Cultures

The grade IV GBM cell line (ACBT) used in this study was a generous gift of G. Granger (University of California, Irvine, USA). The cells were cultured in DMEM (Gibco, Grand Island, NY) with high glucose and supplemented with 2 mM L-glutamine, penicillin (100 U ml\(^{-1}\)), streptomycin (100 µg ml\(^{-1}\)), and 10% heat-inactivated fetal bovine serum (Gibco). Cells were maintained at 37°C in a 7.5% CO\(_2\) incubator. At a density of 70% confluence, cells were removed from the incubator and left at room temperature for approximately 20 minutes. The resultant cell clusters (consisting of approximately 10 cells) were transferred to a petri dish and grown to tumor spheroids of varying sizes. Spheroids were grown according to standard techniques \([17]\). Spheroids of 250 µm diameter were selected by passage through a screen mesh (Sigma, St. Louis, MO). It took approximately 14 days for spheroids to reach a size of 250 µm. The spheroid culture medium was changed three times weekly.

PDT Treatments

Spheroids were incubated in either 100 or 1000 µg ml\(^{-1}\) ALA (Sigma) for approximately 4 hours. In all cases, spheroids were irradiated with 635 nm light from an argon ion-pumped dye laser (Coherent, Inc., Santa Clara, CA). Light was coupled into a 200 µm diameter optical fiber containing a microlens at the output end. Spheroids were irradiated in a petri dish. A 2 cm diameter gasket was placed in the dish to confine the spheroids to the central portion of the dish and thus limit the extent of the irradiated field. Following irradiation, individual spheroids were placed into separate wells of a 64-well culture plate and monitored for growth. Spheroid sizing was carried out by measuring two perpendicular diameters of each spheroid using a microscope with a calibrated eyepiece micrometer. Typically, 10–12 spheroids were followed for each irradiation condition. Since each trial was performed three or four times, a total of 30–50 spheroids were followed for a given set of parameters. Spheroids were followed for up to 45 days.

In the case of the fluence/fluence rate studies, spheroids were incubated in 1000 µg ml\(^{-1}\) of ALA and subjected to a fluence of either 25 or 50 J cm\(^{-2}\) delivered at fluence rates of 5, 10, 25, 50, 75, 150, or 200 mW cm\(^{-2}\). In the repeat PDT studies, spheroids were grown in petri dishes (40–50 spheroids per dish). One of the dishes received no treatment and served as the control. Two groups of spheroids were treated once using fluences of either 12 or 25 J cm\(^{-2}\). A third group was treated three times \((12 + 12 + 25)\) J cm\(^{-2}\) at weekly intervals. In all cases, a fluence rate of 25 mW cm\(^{-2}\) was used. Spheroids were incubated in 100 µg ml\(^{-1}\) of ALA prior to each treatment. Following treatment, spheroids were washed and resuspended in medium. At the conclusion of the last irradiation, spheroids were removed from the petri dishes and placed into separate wells of a 64-well culture plate where they were monitored for growth.

Model of Light Distribution in Brain Tissue

In scattering media, such as biological tissue, light distributions can be adequately described by diffusion theory. In the case of a spherical applicator positioned in the center of a spherical cavity, the fluence rate is given by \([18]\):

\[
\varphi = \frac{Pc}{4\pi aDr^2} e^{-\alpha r} = \frac{Pc}{4\pi a Dr^2} e^{-\alpha r/\delta},
\]

where \(P\) is the total optical power from the applicator, \(c\) is the velocity of light in tissue, \(\alpha\) is the applicator radius, \(r\) is the distance from the applicator center, \(\delta\) is the optical
penetration depth, and $D$ is the diffusion constant. The optical dose at a particular depth is given by:

$$\Psi = \varphi \cdot t$$

(2)

where $t$ is the irradiation time. Since decomposition of the photosensitizer (photobleaching) is common during PDT, an effective dose can be specified:

$$\Psi_{\text{eff}} = \Theta (1 - e^{-\Psi_t})$$

(3)

where $\Theta$ is the photobleaching parameter.

RESULTS

Fluence and Fluence Rate Effects

Effects of fluence and fluence rate on spheroid survival are illustrated in Figure 1. Spheroids are assumed to have survived treatment if they grow at any time during the observation period. Three different control groups were monitored: (i) true controls (no light, no photosensitizer), (ii) light only, and (iii) photosensitizer only. In all cases, 100% survival was observed. As shown in Figure 1, spheroid survival is sensitive to both fluence and fluence rate. The data indicate that, over fluence rates ranging from 10 to 200 mW cm$^{-2}$, lower fluence rates are more effective than higher ones. In other words, the threshold fluence can be decreased simply by irradiating over a longer period of time, i.e., by lowering the fluence rate. As shown in Figure 1, there is a fluence rate threshold (ca. 10 mW cm$^{-2}$) below which the surviving fraction increases. At all fluence rates investigated, the higher fluence (50 J cm$^{-2}$) was more effective.

Repeated PDT Treatments

The effects of repeated weekly PDT treatments are illustrated in Figure 2. Each group of spheroids was irradiated on Day 0. In addition, the multiply treated group was irradiated on Days 7 and 14. Spheroids in all three groups were transferred from petri dishes to individual well plates on Day 14. As shown in Figure 2, spheroid survival is strongly dependent on light dose. Sub-threshold fluences of 12 and 25 J cm$^{-2}$ yield 100 and 61% spheroid survival, respectively. In contrast, only 25% of the spheroids treated three times (with sub-threshold fluences) show growth potential. No growth is observed in the multiply treated spheroids as long as treatment is continued. All three control groups (light only, ALA only, and no light, no ALA) exhibited growth throughout the entire observation period (data not shown).

Applicator Design

As illustrated in Figure 3a,b, the balloon applicator consists of two parts: a two-lumen silicone catheter with an attached inflatable balloon, and a funnel shaped solid plastic insert that fits into the central lumen tip and supports a self-sealing penetrable membrane. The optical fiber is inserted into the central lumen and the balloon is inflated via injection of liquid into the smaller filling lumen. The distal end of the central lumen is sealed just below the end of the balloon with a transparent silicone plug. The end of the balloon-filling channel, residing in the catheter wall, is also sealed off to prevent leakage and balloon deflation. The length of the catheter is determined during the surgical implantation and must be amenable to in situ determination and modification, this is true of both the central lumen and the balloon inflating lumen. The membrane support insert is funnel shaped to allow ease of penetration through the skin and guides the optical fiber into the central lumen in a non traumatic fashion so as not to damage the fiber. The distal end of the membrane support insert is placed into the end of the central lumen,
thus expanding the catheter and resulting in a snug fit. The insert sits on the cranial bony surface so that no forces are transmitted into the brain. The entire device is covered by intact skin thus preserving sterility and greatly reducing the risk of infection. Since the central lumen is sealed at both ends, it has no contact with brain, CSF, or other biological tissue or fluids.

Following implantation and wound healing, the top of the device is palpated through the skin and both the skin and applicator membrane are punctured with a needle with mandrill. The mandrill is removed and the optical fiber is threaded down the center of the balloon. The fiber and needle are withdrawn after treatment. At the termination of treatment, the applicator is removed by making a small skin incision under local anesthesia. The balloon is deflated and the entire device is withdrawn.

**Calculated Light Distribution in Brain Tissue**

Equation 1 is used to estimate dose rates in brain tissue for the range of applicator sizes typically used in the clinic (Fig. 4). The calculations assume an input power of 1 W ($\lambda = 630$ nm), an optical penetration depth of 3.2 mm [19–21], and a diffusion constant of $5.4 \times 10^{10}$ mm$^2$ sec$^{-1}$ [19–21]. Figure 4 illustrates a fundamental limitation of PDT, namely the poor penetration of light in brain tissues. For example, fluence rates decline by three to four orders of magnitude over tissue depths of 2 cm. The protective effect of photobleaching is illustrated in Figure 5. The fluence ($\Phi$) and effective fluence ($\Phi_{\text{eff}}$) are calculated from Equations 2 and 3, respectively. The calculations assume a 3.0 cm (diameter) applicator and an input power of 1.0 W—significantly below the power required for the induction of thermal effects [22]. A 5000 second irradiation time is chosen since this is the time required to deliver a threshold fluence of 50 J cm$^{-2}$, at a fluence rate of 10 mW cm$^{-2}$. A 50 J cm$^{-2}$ bleaching parameter is used in the calculation as this is representative of ALA [23].

**DISCUSSION**

The results of the in vitro studies presented here suggest that repeated PDT treatments are more effective than commonly used “one-shot” procedures. In addition, light...
doses are more effective if delivered at lower dose rates. These findings provide the rationale for the development of an indwelling balloon applicator that provides great flexibility for light delivery during PDT—light doses can be delivered for prolonged time periods, and at any time following surgery. The applicator allows for optimization of light delivery thus increasing the likelihood of a successful treatment outcome.

The observation that lower fluence rates are more effective than higher ones agrees with the findings of others using different spheroid models [24]. Theoretical models suggest that the spatial distribution of singlet molecular oxygen (the primary cytotoxic species) depends critically on the fluence rate [25]. At a given spheroid depth, the concentration of singlet molecular oxygen increases as fluence rates decrease. As a result, photodynamic damage will extend further into the spheroid as the fluence rate is lowered. Thus, PDT administered at lower fluence rates yields improved therapeutic response since singlet molecular oxygen is delivered to a larger volume of tumor cells in the spheroid. The results of the present study suggest that the minimum effective fluence rate is approximately 10 mW cm\(^{-2}\). The calculations illustrated in Figure 4 show that, for an input power of 1 W, the 10 mW cm\(^{-2}\) threshold occurs at depths ranging from approximately 0.9 to 1.2 cm in typical brain tissue. Although the treatment of larger volumes is possible simply by increasing the input power, one must be careful to avoid thermal effects. Calculations show that the induction of hyperthermia limits treatment depths in brain tissue to approximately 1.4 cm [22], although it may be possible to treat to depths of 1.8 cm by cooling the cavity. This could be accomplished by circulating water through the applicator.

At a fluence rate of 10 mW cm\(^{-2}\), the delivery of an optical dose of 50 J cm\(^{-2}\) requires 1.4 hour. The clinical finding that higher fluences (>100 J cm\(^{-2}\)) are associated with improved PDT response, suggests that treatment times of the order of several hours may be required [10]. Such long treatments are impractical in an intraoperative setting, but are readily possible using an indwelling balloon applicator.

The results of PDT fractionation studies involving fractionation intervals of days to weeks are somewhat mixed; some show favorable responses [26,27], while others are inconclusive [28]. The results presented here indicate that weekly repeated PDT treatments using sub-optimal fluences are very effective at suppressing spheroid growth (Fig. 2). Although the explanation of these results is unclear, the effects of fractionation and repeated PDT may be due to the same mechanism underlying the effectiveness of fractionation with ionizing radiation, i.e., when layers of cells near the surface of the spheroid are irreversibly damaged, these cells no longer utilize oxygen thus allowing oxygen to become available via diffusion to cells located deeper in the spheroid. In this way, successively deeper layers of cells become oxygenated and the resultant cytotoxicity advances deeper into the spheroid with every light fractionation.

The clinical utility of daily PDT fractionation is uncertain due to systemic toxicity associated with frequent administration of ALA [29]; however, weekly or himonthly treatments are readily possible. The results of the weekly PDT treatments are especially interesting since they show that fractionation with sub-threshold fluences not only reduces spheroid survival, but also suppresses growth of surviving spheroids. As illustrated in Figure 2, growth is only suppressed during the treatment period—re-growth is observed approximately 1 week following the last treatment. This clearly demonstrates the importance of repeated access to the tumor resection cavity over an extended time frame allowing multiple treatments. The indwelling balloon applicator was developed specifically to take advantage of the enhanced efficacy of repeated PDT treatments over extended time periods.

Although balloon-type applicators have been used previously to optimize light dose distributions during intraoperative PDT of the brain [30], the applicator described here is, to our knowledge, the first designed for long-term implantation. The applicator is a modification of a pre-existing device currently used in afterloading brachytherapy to deliver high doses of ionizing radiation to resected tumor margins during the first post operative week [31]. Although the suitability of the brachytherapy applicator for PDT was confirmed in a previous study [32], it is unlikely that it can be used in long-term PDT treatments due to its partially explanted nature and subsequent concerns of infection—the applicator protrudes approximately 1 cm from the skull surface. The modified applicator addresses the problem of sterility as it is entirely covered by intact skin and, due to the reduced risk of infection, it is ideally suited to long-term repeated PDT treatments of several months duration or longer. The applicator has the following novel features: (1) it defines the cavity to be irradiated; (2) it provides uniform light distribution to surrounding tissues; (3) it is completely implanted and does not penetrate the skin; (4) it allows
repeated access to the cavity to be irradiated over long time periods via simple skin puncture; (5) it can be modified to allow direct delivery of photosensitizer to the target tissue, and (6) it can be removed in a relatively simple, non-traumatic manner.

Although the balloon applicator is an ideal light delivery device, it should be noted that, in addition to the light dose, the overall efficacy of PDT depends in a complex manner on such factors as the kinetics of photosensitizer uptake, cellular localization, and tissue oxygenation status. Although, in theory, it may be possible to treat to depths of approximately 1.5 cm in brain tissue, the high light doses in tissues near the applicator may result in unacceptable damage to healthy tissues. This is especially true in the case of poorly localizing photosensitizers. However, as illustrated in Figure 5, the optical dose is reduced drastically in the high dose region near the applicator due to the effects of photobleaching. The calculations show that, for a bleaching parameter of 2, photobleaching lowers the optical dose by approximately 1.5 orders of magnitude in tissues adjacent to the applicator (Fig. 5).

In summary, a relatively simple in vitro spheroid model has been used to optimize light dose delivery for ALA-mediated PDT. The results suggest an enhanced benefit of both low fluence rates and long-term repeated PDT treatments thus providing the rationale for the development of an indwelling balloon applicator. Although intracranial applications were the main motivation for this study, the concepts developed here can be used at other sites in the body.

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