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Fluence rate effects in human glioma spheroids: implications for photodynamic therapy of brain tumors

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ABSTRACT

The effects of fluence rate are investigated in human glioma spheroids incubated in 5-aminolevulinic acid (ALA). It is shown that the response of glioma spheroids to ALA-mediated PDT depends strongly on the rate at which the light dose is delivered. At low doses, lower fluence rates are more effective. For example, at a dose of 50 J/cm^2, near total spheroid kill is observed at fluence rates of as low as 10 mW/cm^2. Below 10 mW/cm^2, however, treatments are not as effective. The fluence rate effect is not as pronounced at higher doses where a favorable response is observed throughout the range of fluence rates investigated. The clinical implications of these findings are discussed.

Keywords: Photodynamic therapy, 5-aminolevulinic acid, glioma spheroids, brain tumor, fluence rate

1. INTRODUCTION

The poor prognosis for patients with high grade gliomas has led to a search for better treatments. Failure of standard treatment regiments (surgery, chemotherapy and ionizing radiation) is usually due to local recurrence at the site of surgical resection indicating that a more aggressive local therapy could be of benefit. A number of studies have shown that photodynamic therapy (PDT) may prove to be useful in prolonging survival of patients with high grade gliomas, such as glioblastoma multiforme[1-7].

Porphyrins, such as hematoporphyrin derivative and Photofrin® have been used almost exclusively in clinical PDT trials of the brain. Although favorable results have been reported by a number of clinicians[8,9], these photosensitizers have several drawbacks that may limit their usefulness. These include long lasting cutaneous photosensitization following systemic administration, and poor tumor-to-normal tissue localization that may result in treatment limiting normal tissue complications[10-12]. Due to the drawback of traditional porphyrins, other photosensitizers, such as 5-aminolevulinic acid (ALA), are currently being evaluated for use in PDT of gliomas.

ALA has been used primarily as a topical agent in the treatment of superficial skin lesions[13], 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 the treatment of a wide variety of lesions[14]. ALA has a number of features that make it an attractive photosensitizer for use in the type of fractionated and/or repeated PDT treatment regimens that may be required to achieve local control in patients with high grade gliomas. For example, some animal studies indicate that ALA has better tumor-to-normal brain tissue localization than traditional photosensitizers[11]. In addition, ALA can be administered orally, and the associated skin photosensitization lasts for a relatively short period of time (24 to 48 h).

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Due to the highly attenuating nature of brain tissue, long treatment times are required to deliver sufficient light doses to cm depths in the resection margin. Furthermore, a number of *in vitro*[15,16] and *in vivo*[17-20] studies suggest that response to PDT depends not only on total fluence , but also on the rate at which the fluence is delivered – lower fluence rates appear more efficacious in many instances.

In this paper, the response of human glioma spheroids to ALA PDT is investigated. Of particular interest is the response of spheroids to the low fluence rates observed in the resected tumor margin during typical PDT treatments. To this end, spheroid survival and growth were monitored as functions of fluence and fluence rate.

2. MATERIALS AND METHODS

2.1 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), streptomycin (100 μg/ml), and 10 % heat-inactivated fetal bovine serum (Gibco, Grand Island, NY). Cells were maintained at 37 ^{0}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 (32). Spheroids of 250 μm diameter were selected by passage through a screen mesh (Sigma, St. Louis, MO). It took approximately 14 days for the spheroids to reach a size of 250 μm. The spheroid culture medium was changed three times weekly.

2.2 PDT Treatments

Spheroids were incubated in 1000 μg ml^{-1} of ALA (Sigma, St. Louis, MO) 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 dia. optical fiber containing a microlens at the ouput end. Spheroids were exposed to fluences of 25, 50, 100 or 200 J cm^{-2} delivered at fluence rates of 5, 10, 25, 50, 75, 150 or 200 mW cm^{-2}. 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 96-well culture plate and monitored for growth. A microscope with a calibrated eyepiece micrometer was used to measure spheroid diameter. Determination of spheroid size was carried out by measuring two perpendicular diameters of each spheroid using a microscope with a calibrated eyepiece micrometer. Typically, 10 to 12 spheroids were followed for each irradiation condition. Since each trial was performed 3 or 4 times, a total of 30 to 50 spheroids were followed for a given set of parameters. Spheroids were followed for up to 35 days.

3. RESULTS

Effects of light fluence and fluence rate are illustrated in Figs. 1a and b. At low fluences (≤ 50 J cm^{-2}; Fig. 1a), spheroid survival is very sensitive to both fluence and fluence rate. At higher fluences (Fig. 1b), the fluence rate dependence is minimal – significant spheroid kill is observed at all fluence rates. This is especially the case at the highest fluence investigated (200 J cm^{-2}). As the total fluence is decreased, however, the effects of fluence rate become more pronounced. At lower fluences (Fig. 1a), it appears that lower fluence rates are more effective than higher ones. It thus appears that the threshold light dose can be decreased simply by giving the dose over a longer period of time, i.e., by lowering the fluence rate. There appears to be a threshold fluence rate below which survival increases. For example, for a fluence of 50 J cm^{-2}, less than 5 % of spheroids survive a fluence rate of 10 mW cm^{-2}, however, lowering the fluence rate to 5 mW cm^{-2} results in 38 % spheroid survival. The results presented in Fig. 1a indicate that, even at optimal fluence rates (25 mW cm^{-2}), a minimum fluence of 50 J cm^{-2} is required in order to achieve 100 % spheroid kill. To achieve a comparable effect at a fluence rate of 50 mW cm^{-2} would require a total fluence

of between 150 and 200 J cm^{-2} (Fig. 1b). At fluence rates of 75 mW cm^{-2} and higher, total spheroid kill could not be achieved, even at the highest fluence (200 J cm^{-2}). To avoid hyperthermic effects, fluences in excess of 200 J cm^{-2} were not attempted.

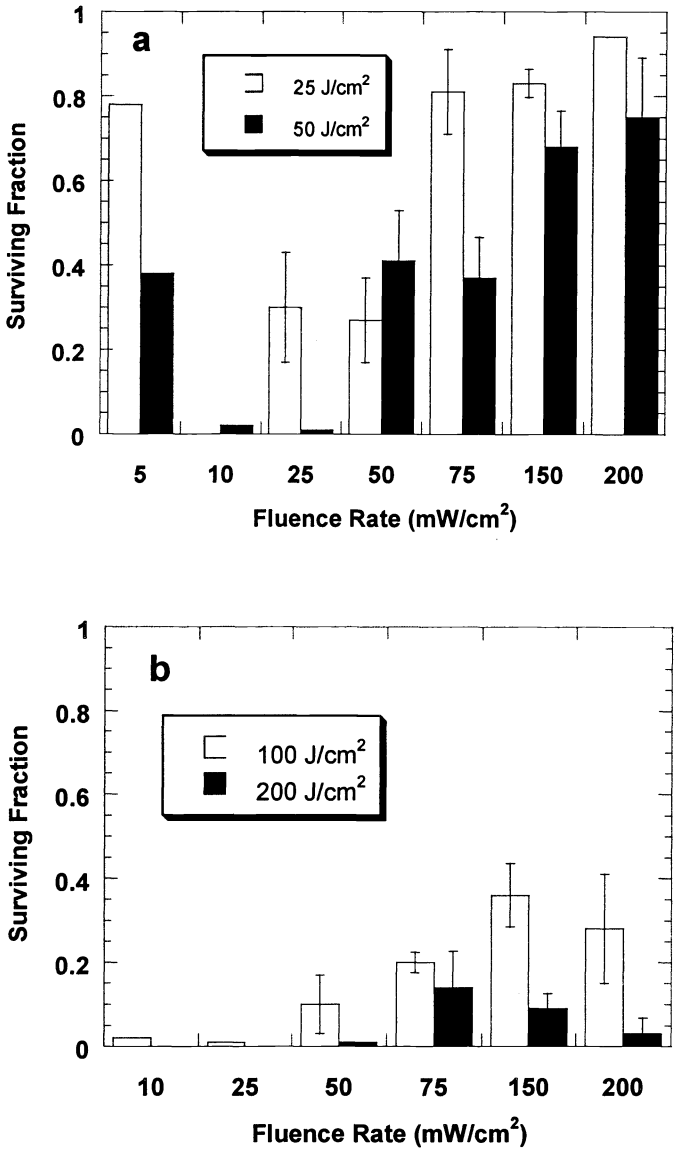

Figure 1. Spheroid survival as a function of fluence rate at representative fluences: (a) 25 and 50 J cm^{-2}; and (b) 100 and 200 J cm^{-2}. Each data point represents the mean of approximately 40 spheroids. Standard errors are denoted by error bars.

The effect of fluence rate on growth delay is illustrated in Fig. 2. Each data point represents the mean diameter of spheroids surviving a particular PDT treatment. The figure shows that there is a dose-rate dependent growth delay: longer growth delays are observed at the lower fluence rates. For example, at a fluence rate of 25 mW cm^{-2}, it takes approximately 15 days to reach a diameter of 500 μm, while at a fluence rate of 200 mW cm^{-2}, this size is attained after only 9 days.

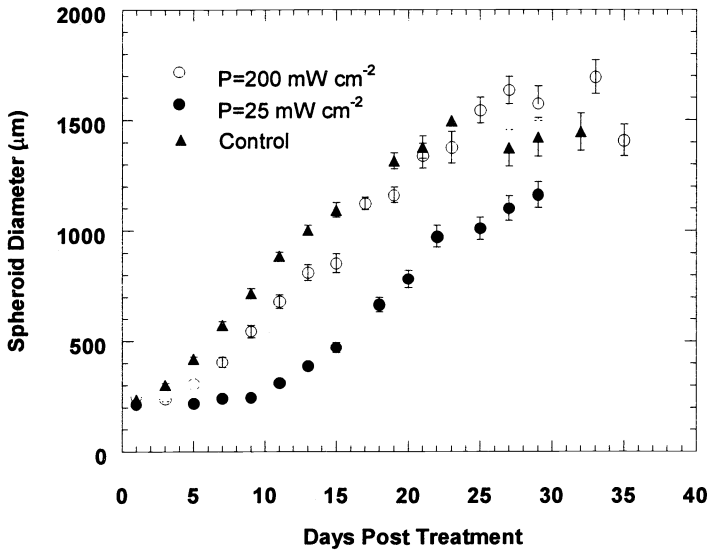

Figure 2. Growth kinetics of spheroids exposed to 25 J cm^{-2} delivered at fluence rates of 25 and 200 mW cm^{-2}. Each data point represents the mean of approximately 40 spheroids. Standard errors are denoted by error bars.

Figure 3 shows that, for a particular fluence rate, there is a fluence-dependent growth delay; higher fluences result in significantly longer delays than lower ones. Interestingly, terminal spheroid size appears to depend on fluence as well. Spheroids exposed to 200 and 25 J cm^{-2} reach terminal sizes of approximately 700 and 1500 µm, respectively.

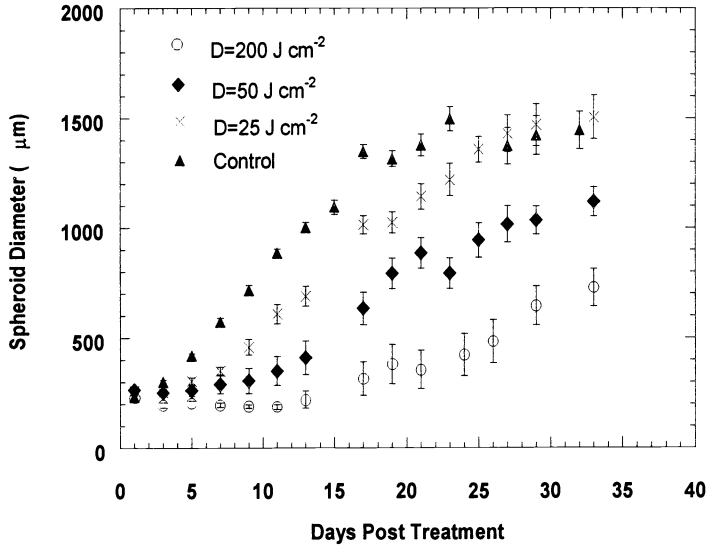

Figure 3. Growth kinetics of spheroids exposed to fluences of: 200, 50 and 25 J cm^{-2}. A fluence rate of 75 mW cm^{-2} was used in all cases. Each data point represents the mean of approximately 40 spheroids. Standard errors are denoted by error bars.

4. DISCUSSION

The efficacy of PDT depends on a number of factors, including the rate of light dose delivery. The results of this study are in good agreement with the findings of other investigators who have observed enhanced photodynamic response at low dose rates using different spheroid models[21]. Theoretical models suggest that the spatial distribution of singlet molecular oxygen depends critically on the dose rate[22]. At a given spheroid depth, the concentration of singlet molecular oxygen increases as dose rates decrease. As a result, photodynamic damage will extend further into the spheroid as the dose rate is lowered. Thus, PDT administered at lower dose rates yields improved therapeutic response since singlet molecular oxygen is delivered to a larger volume of tumor cells in the spheroid.

Preliminary results of ongoing clinical trials in patients with supratentorial gliomas suggest that relatively high light doses (ca. 80 J cm^{-2}) are required for prolongation of survival[23]. The results of the present study show that the delivery of doses between 50 and 100 J cm^{-2} requires low dose rates for optimum PDT response. Unfortunately, the delivery of high doses (at low dose rates) to tumor cells at cm depths in the resection margin, requires treatment times (hours) which are typically much longer than those tolerated in standard one-shot intraoperative procedures. To address this problem, an indwelling balloon applicator has been developed[24]. The applicator is inserted into the resection cavity following surgical debulking and can remain there for long periods of time (months to years). The applicator provides great flexibility in light dose delivery and facilitates investigation of different light delivery schemes (e.g. long-term low dose rates, fractionation, and repeated treatments). The applicator has already been used for high dose rate brachytherapy in over 100 patients at Rikshospitalet in Oslo, Norway[24].

Due to its rapid skin clearance, and possibility of oral administration, ALA is ideally suited to clinical studies investigating different light delivery schemes. The combination of ALA and novel light delivery techniques may result in improved PDT outcomes in patients with high grade gliomas.

5. CONCLUSIONS

The response of human glioma spheroids to ALA-mediated PDT is critically dependent on light dose and dose rate. The results of the present study suggest that lower dose rates result in enhanced photodynamic effect as evidenced by decreased spheroid survival and increased growth delay. This is especially true for light doses $\leq$ 50 J cm^{-2}. At higher doses, the dose rate effect is not as pronounced. The results suggest that there is a threshold dose rate below which the effects of PDT diminish. The data indicate that, for a dose of 50 J cm^{-2} the threshold dose rate is between 6 and 9 mW cm^{-2}. The validity of extrapolating the *in vitro* results to the clinic is unknown, however, the findings of this study suggest that the delivery of adequate light doses to the resection margin may be difficult to achieve with current treatment techniques. A possible alternative is the use of indwelling balloon applicators that would facilitate the delivery of light over long periods of time, i.e., at low dose rates.

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