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5-Aminolevulinic Acid Photodynamic Therapy for the Treatment of High-Grade Gliomas

K Mahmoudi[1], **KL Garvey**[1], **A Bouras**[2], **G Cramer**[4], **H Stepp**[5], **JG Jesu Raj**[2], **D Bozec**[2], **TM Busch**[4], and **CG Hadjipanayis**[2,3]

[1]Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[2]Brain Tumor Nanotechnology Laboratory, Department of Neurosurgery, Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

[3]Department of Neurosurgery, Mount Sinai Beth Israel, New York, NY, USA.

[4]Department of Radiation Oncology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA.

[5]Laser-Research Laboratory, LIFE-Center at the Department of Urology, University Hospital of Munich, Munich, Germany.

Abstract

Photodynamic therapy (PDT) is a two-step treatment involving the local administration of a photosensitive agent followed by its activation at a specific light wavelength. PDT has been approved by the United States Food and Drug Administration (FDA) for the treatment of premalignant and malignant diseases, such as actinic keratoses, Barrett's esophagus, esophageal cancers, and endobronchial non-small cell lung cancers, as well as for the treatment of choroidal neovascularization. In oncology, clinical trials are currently underway to demonstrate PDT efficacy against a number of malignancies that include glioblastoma (GBM) and other brain tumors. Both photosensitizers and photosensitizing precursors have been used for PDT. Photofrin and Visudyne are photosensitizers with FDA approval for PDT of high-grade dysplasia in Barrett's esophagus and subfoveal choroidal neovascularization, respectively. 5-aminolevulinic acid (5-ALA), an intermediate in the heme synthesis pathway, is a photosensitizing precursor with FDA approval for PDT of actinic keratosis and fluorescence-guided visualization of malignant tissue during glioma surgery. In this review, the history and current use of 5-ALA PDT for the treatment of high-grade gliomas (HGGs) will be discussed.

Keywords

5-Aminolevulinic Acid (5-ALA); Photodynamic Therapy; GBM; Protoporphyrin IX (PpIX); High Grade Glioma; Photosensitizer

Constantinos G. Hadjipanayis (constantinos.hadjipanayis@mountsinai.org); Theresa M. Busch (co-senior; buschtm@pennmedicine.upenn.edu).

Introduction

According to the Central Brain Tumor Registry of the United States (CBTRUS), the incidence of brain tumors, both malignant and non-malignant, in adolescent and young adult patients (15 – 39 years old) is 10.71 per 100,000 and in those over 40 years of age is 40.10 per 100,000 [1, 2]. High-grade gliomas (HGGs) are defined by the World Health Organization (WHO) as grade 3 (anaplastic tumors) and grade 4 (mainly glioblastoma (GBM)) brain tumors [3]. The incidence of HGGs is approximately 5 per 100,000, with a peak incidence in patients between 50 and 69 years of age [4]. The standard of care for HGGs includes surgical resection, when possible, followed by chemotherapy, and radiation therapy (RT) [5]. The median 2- and 5-year survival for patients with anaplastic astrocytoma (AA) is approximately 50% and 24%, respectively, and for patients with GBM is approximately 27% and 10%, respectively [6-8].

The poor outcomes of patients with HGGs have driven the exploration of novel adjuvant therapies. These therapies, such as immunotherapy, alternating electric tumor treating fields, laser-induced interstitial thermotherapy (LITT), magnetic hyperthermia therapy (MHT), focused ultrasound, radiofrequency microwaves, and photodynamic therapy (PDT) have demonstrated variable levels of success [9-13].

Photodynamic Therapy

PDT was first described by the inhabitants of ancient Greek, Egyptian, and Indian civilizations [14]. These civilizations believed light alone was sufficient for the treatment of certain diseases, including vitiligo, rickets, psoriasis, and skin cancer. However, it was not until the twentieth century that a photosensitizing agent was incorporated with the use of light for therapeutic purposes. PDT involves intravenous (IV), intraperitoneal (IP), topical, or oral delivery of a photosensitive agent that generates cytotoxic reactive oxygen species (ROS) during its activation at a specific light wavelength. Fluorescence of the photosensitizer can be observed after direct exposure of the compound to the appropriate activating wavelength [15]. Most commonly used photosensitizers are dependent on tissue concentrations of molecular oxygen, due to the role of singlet oxygen and other ROS in the tissue damage inflicted by PDT. This damage can manifest in necrosis and apoptosis of tumor cells, as well as in the destruction of tumor vasculature [12, 16]. The short distance of migration for singlet oxygen (i.e. ~0.02 – 1 μm) and its short lifespan (i.e. ~0.04 – 4 μs) allows PDT to primarily damage tumor cells while sparing adjacent normal tissues [12, 15, 16]. Notably, this effect depends on the observed differences in selectivity and uptake of the photosensitizer in tumor versus healthy cells. The United States Food and Drug Administration (FDA) has approved PDT for applications in oncology and other diseases (e.g., as an adjuvant treatment modality for esophageal and non-small cell lung cancers, for treatment of premalignant conditions such as Barrett's esophagus and actinic keratoses, and for treatment of subfoveal choroidal neovascularization due to age-related macular degeneration, pathologic myopia, or presumed ocular histoplasmosis) [17, 18].

PDT Mechanisms of Cell Death

Many mechanisms of cytotoxicity have been described with PDT, including the activation of cell death and survival pathways as constituted by necrosis, apoptosis, autophagy, necroptosis, and paraptosis [19]. Cell necrosis is generally associated with damage introduced at higher PDT doses, while apoptosis may be associated with cytochrome c release, Bcl-2 photodamage, and NFκB suppression [20, 21]. For example, in a study of HGGs, PDT induced a pro-apoptotic response as characterized by an increase in the Bax:Bcl-2 ratio and downregulation of NFκB in U87MG GBM cells [22]. A different group has reported induction of RIP3-dependent necrosis in LN18 GBM cells following PDT [23]. Moreover, this same group has also described NFκB inhibition leading to necrosis of LN18 and U87MG HGG cells after PDT [24]. In addition to its direct cytotoxic effects, PDT can destroy tumor microvasculature with the potential to starve remaining tumor cells of essential oxygen and nutrients [25]. Moreover, PDT can lead to immunogenic cell death with the potential to stimulate immune activation and surveillance, contributing to long term tumor control [26-28].

Immunomodulatory Effects of PDT

PDT has a significant effect on the immune system, largely due to its capacity to increase the host immune response against cancer [29-31]. PDT-induced cell damage releases a mixture of tumor antigens and other signals which activate both the innate and adaptive immune systems [29]. *In vitro*, Etminan et al. found that 5-ALA PDT of GBM spheroids recruits and activates dendritic cells, which importantly speaks to an immunomodulatory role for 5-ALA PDT in the stimulation of antigen presenting cells [32]. *In vivo*, PDT generated regional and systemic anti-tumor immunity in mice with G422 HGGs; these rodents demonstrated increased infiltration of immune cells and cytokine release following PDT compared to controls [33]. Further evidence of PDT-mediated immune effects in the treatment of HGGs is found in the work of Li et al., albeit with a hematoporphyrin derivative as the photosensitizer. They showed that PDT of murine G422 GBM cells led to tumor infiltration by immune cells that is accompanied by release of cytokines IFNγ and TNFα; splenocyte transfer from immunocompetent PDT-treated glioma-bearing mice could suppress glioma growth in recipient immunosuppressed animals thereby indicating that PDT led to the generation of systemic anti-tumor immunity [33].

Light Wavelength and Penetration Depth

In general, the range of wavelengths used for PDT has been ~400 – 900 nm, with the preferred therapeutic window (600 – 800 nm) based on the goals of minimizing light absorption by tissue chromophores (water, hemoglobin, melanin, etc.) and achieving a sufficiently energetic excited state photosensitizer that will produce singlet oxygen upon interaction with molecular oxygen [34]. Over the commonly used wavelength range of 630 – 690 nm, reported values for light penetration in various types of tissue fall mainly between 1 – 5 mm, with slightly greater penetration at higher wavelengths [35, 36]. Thus, the majority of light energy is lost within the first 5 mm, with minimal amounts reaching 1 cm in depth [37].

Penetration depth is defined as the distance into tissue at which the intensity of applied light is reduced to approximately 37%. It is dependent on factors such as reflection, scattering, and absorption of light in tissue [37]. Notably, however, the effective depth of PDT damage is greater than light penetration depth and can extend to distances of 1 cm or greater [37]. Consequently, PDT is most promising for eradication of superficial tumors, including early stage or premalignant disease, and residual tumor cells left behind after surgical resection that are often responsible for recurrence [38, 39]. Moreover, in certain scenarios, light delivery into deeper tissues or larger tumors can be achieved via interstitial PDT incorporating intratumoral placement of fiber optic light delivery devices [38, 40, 41].

PDT Light Delivery and Fluorescence Imaging

Intraoperative devices have been developed specifically for PDT of GBM. Light can be delivered to the tumor bulk through the interstitial placement of fibers or to the resection cavity after surgery. For the post-surgical approach, a device with an inflatable balloon filled with diffusing liquid coupled to a trocar with an optical fiber guide has been developed [42]. The device is guided inside the resection cavity, filled with fluid until the balloon wall reaches the boundaries of the cavity, and illuminated. The safety of this device is currently being evaluated in the Intraoperative Photodynamic Therapy of GBM (INDYGO) clinical trial [43]. Light delivery protocols for PDT of GBM (as for other diseases), include the use of low fluence rate or fractionated illumination as means to preserve molecular oxygen for the photochemical reaction [44]. Moreover, in a metronomic approach, preclinical studies have evaluated low doses of both photosensitizer and light over periods of prolonged exposure as a means to provide for a more selective response [45]. With deep or otherwise inoperable tumors, cylindrical diffuser fibers can be inserted into the tumor to deliver the light. This approach requires careful planning for safe placement of the light fibers and reliable irradiation of the entire tumor [46-48].

Photosensitizing agents have also been employed independent of PDT to assist in the delineation of tumor margins for maximal tumor resection during fluorescence-guided surgery (FGS) [49]. FGS permits direct visualization of tumor tissue for real-time intraoperative surgical guidance independent of brain shift [50]. Various agents tested for FGS have included 5-ALA, fluorescein, indocyanine green, and endogenous fluorophores [51]. 5-ALA for FGS (Gleolan©) has recently been approved by the FDA for intraoperative visualization of malignant tissue [52]. Other conventional imaging methodologies, including computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and ultrasound, provide valuable information intraoperatively. However, these imaging modalities exhibit a variety of limitations, including poor tissue contrast and spatial resolution, cost, length of time added to surgery, and safety concerns [53].

Photosensitizers

The ideal photosensitizer for application in brain tumors must be systemically non-toxic, accumulate in high concentrations in the tumor tissue, and be activated at wavelengths of light sufficient for deep brain tissue penetration, while nontoxic to the surrounding brain [37]. A number of different photosensitizers or photosensitizing precursors, including

hematoporphyrin derivative (HpD), porfimer sodium (Photofrin), temoporfin, verteporfin, and 5-ALA have been used clinically to treat different brain tumors, including HGGs [44].

There are currently three generations of photosensitizers, with HpD and Photofrin the first to be utilized for PDT [54]. Photofrin was first approved by the FDA as a PDT agent in 1995 as a palliative therapy for obstruction relief in patients with esophageal cancer [55]. Second generation photosensitizing agents include a broad spectrum of drugs with a better defined chemical composition than first generation sensitizers. These include porphyrins, chlorins, pheophorbides, bacteriopheophorbides, metalloporphyrins, purpurins, and phthalocyanines [56, 57]. Also classified as a second-generation photosensitizer, 5-ALA or its ester derivatives are used as prodrugs because they act as photosensitizer precursors that are metabolized to the endogenous photosensitizer protoporphyrin IX (PpIX). Boronated porphyrins (BOPP) are another second-generation photosensitizer of particular mention for brain applications. BOPP, combined with PDT and boron neutron capture therapy, has the potential to provide for multi-modality treatment of brain malignancy that is sensitized by the administration of a single drug [58]. As a PDT photosensitizer, BOPP is reported to require less light energy than HpD to mediate tumor cell death [59]. It belongs to a subcategory of second-generation photosensitizers that localize primarily within the tumor cell mitochondria [60].

Third generation photosensitizers demonstrate enhanced tumor targeting capabilities compared to older generations as they are designed to bind with high specificity to tumor cells [61]. This generation can be separated into three broad categories: PDT nanotechnology, gene engineering-mediated PDT, and carrier-bound PDT [54, 62]. Third generation photosensitizers that have been studied for PDT of brain tumors include photosensitizer-carrying nanoparticles conjugated to molecules that facilitate tumor targeting. For example, the F3 peptide has been used to target tumor cells and angiogenic vasculature, while a peptide motif that targets neuropilin-1, a receptor for vascular endothelial growth factor, has been used to direct PDT against neo-angiogenic blood vessels [63-65]. An epidermal growth factor peptide has also been exploited in PDT of rodent brain tumors for the delivery of photosensitizer absorbed on the surface of gold nanoparticles [66]. In another approach, Rajora et al. have used apolipoprotein E3 (apoE3) nanoparticles to facilitate photosensitizer delivery and binding to GBM cells [67]. Functionality is provided by the role for apoE3 as a chaperone of cholesterol transit in the brain, as well as its binding to the low density lipoprotein receptors that are overexpressed on GBM cells in response to their increased need for cholesterol. Lastly, several groups have recently explored the use of upconverting nanoparticles for PDT of brain tumors [68, 69]. The underlying premise of this approach is to utilize deeper-penetrating near infrared light to stimulate the release of visible illumination by the upconverting particle, for example, those created by lanthanide doping. Particles are designed to produce the visible illumination at photosensitizer-exciting wavelengths, thus providing the opportunity for more deeply penetrating PDT.

5-Aminolevulinic Acid

5-ALA is a natural intermediate metabolite produced in the hemoglobin metabolic pathway with no inherent fluorescence [53]. After oral administration, 5-ALA can accumulate in

malignant brain tumors and surrounding infiltrating cancer cells outside of the tumor bulk in a process that is facilitated by the increased permeability of tumor-associated vasculature (Figure 1A) [70-72]. Within mitochondria, 5-ALA is converted to PpIX (Figure 1B), which is then acted on by the enzyme ferrochelatase to form heme. Decreased ferrochelatase expression in malignant tissue may contribute to the accumulation of PpIX within gliomas and other neoplastic cells and lead to its preferential localization in tumor (Figure 1B) [73]. Differential expression of the enzyme porphobilinogen deaminase (PBGD) between tumor and normal cells may also contribute to selectivity in PpIX production through its role in catalyzing PpIX biosynthesis [74]. Once formed, PpIX re-emits red fluorescence at a peak wavelength of 635 nm after excitation with light near the Soret band peak (around 410 nm) (Figure 1C), [70, 75]. Additionally, PpIX has 4 absorbance bands between 480 and 650 nm (Q bands) [75]. The fluorescent and photosensitizing properties of 5-ALA/PpIX have been used for treatment of HGGs by both FGS (with blue light activation) and PDT (with red light activation owing to better tissue penetration) [46, 70, 71]. Figure 1E illustrates the absorption spectrum of PpIX.

As topically applied agents, 5-ALA and its methyl ester (methyl-ALA) are FDA approved for PDT of actinic keratosis. 5-ALA can also be administered orally in humans and has been studied in this formulation for PDT of numerous malignancies [76, 77]. It is the only known oral agent available for FGS of HGGs that can accumulate within malignant brain tumors [70]. The red PpIX fluorescence allows for more accurate delineation and differentiation of tumor from normal tissue [53, 70] using surgical microscopes equipped with fluorescence filters [78]. 5-ALA has been approved for FGS of HGGs in the European Union after a landmark randomized, controlled study revealed greater tumor resection and progression-free survival (PFS) with 5-ALA FGS in comparison to conventional microsurgery [49, 79]. 5-ALA was also recently approved by the FDA as the first-ever fluorescing agent for enhanced visualization of malignant tissue during surgical resection of suspected HGGs[52]. 5-ALA-associated tissue fluorescence during FGS has been demonstrated with unprecedentedly high sensitivity, specificity, and positive predictive values for identifying malignant brain tumor tissue [70].

5-ALA PDT

Numerous studies have been performed *in vitro* and *in vivo* that highlight the efficacy of 5-ALA PDT for the treatment of HGGs. Different light and drug exposures have been tested, and the selectivity of PDT for tumor cells versus normal tissue/cells or edematous tissue has been evaluated. In some cases, PDT is performed repetitively, while in others, low fluence rate, fractionated light delivery or combinations of prolonged exposure to photosensitizer and light (metronomic) have been investigated. Studies in animals have tested techniques for light delivery to HGGs, such as illumination of the surgically exposed tumor, as well as interstitial placement of light fibers [42]. Collectively, this body of preclinical work has facilitated the safe translation of 5-ALA PDT into clinical trials.

In Vitro Studies

5-ALA PDT has been explored as a treatment modality for HGGs for the past two decades. The accumulation of PpIX in C6 glioma cells and brain tumors of rats after administration of 5-ALA was noted in 1998 [80]. Some of the earliest *in vitro* studies of 5-ALA PDT of brain tumor cells were published in 1999 [81]. In this study, U-105MG GBM and CH-157MN meningioma cells were incubated with 5-ALA for 24 h, followed by exposure to broadband illumination at 12.4 mW/cm^2, 11 J/cm^2. A MTT assay was used to quantify cell viability at 24-48 h after 5-ALA PDT administration. Increased cytotoxicity of PDT to GBM cells was observed and attributed to preferential accumulation of PpIX within GBM cells compared to meningioma cells.

5-ALA PDT has also been effective in an ACBT human HGG spheroid model [82]. In this study, human HGG spheroids were incubated with 5-ALA for 4 h prior to administration of light at a fluence of either 25 or 50 J/cm^2. Low fluence rates to the same total fluence (requiring longer duration of treatment) resulted in more cell death within the spheroid, which may be a function of the ability for low fluence rates to better conserve oxygen during PDT and maintain the ongoing production of ROS by the photochemical process [83]. Additionally, the effects of multiple PDT sessions were reported, with one experimental group receiving two treatment sessions of 12 J/cm^2 followed by a single session of 25 J/cm^2 over two weeks and the other two groups receiving a single PDT session of either 12 or 25 J/cm^2 [82]. The spheroids treated with multiple PDT sessions had significantly reduced growth potential compared to spheroids treated with a single PDT session. These data serve to highlight the value of continued work on extended periods of light delivery for PDT of brain malignancy.

The *in vitro* cytotoxicity of 5-ALA PDT has also been compared to that of ALA derivatives, including methyl-ALA (m-ALA), hexyl-ALA (h-ALA), and benzyl-ALA (b-ALA) [84]. Human HGG spheroids were incubated in varying concentrations (0.025 – 5.0 mM) of 5-ALA and ALA derivatives for 4 h. 5-ALA and m-ALA (0.05 mM) were similarly cytotoxic after 635 nm illumination (25 J/cm^2, 25 mW/cm^2). However, greater cytotoxicity was achieved with h-ALA and b-ALA under the same conditions. Further comparison of 5-ALA and h-ALA found that PDT with h-ALA achieved a cytotoxic response equivalent to 5-ALA-induced PDT at concentrations 10 – 20 times lower than 5-ALA.

Animal Studies

Studies in animals have evaluated the safety and efficacy of PDT delivery to both normal and malignant brain tissue. Olzowy et al. reported the anti-tumor effect of 5-ALA PDT using an experimental orthotopic rat glioma model [85]. Susceptibility to PDT was compared among three groups: healthy rats, rats with perifocal brain edema, and rats with C6 HGG tumors. Healthy rats received 5-ALA (100 mg/kg) IV followed 6 h later by PDT of the exposed dura and underlying brain at a dose of 200 J/cm^2 (100 mW/cm^2, 635nm). Rats of the edematous brain tissue group received 5-ALA (100 mg/kg) IV followed 3 h later by cold injury to the brain cortex to cause edema. After an additional 3 h, PDT was performed at 200 J/cm^2. In the experimental C6 HGG tumor group, rats received 5-ALA (100 mg/kg) IV followed by PDT after either 3 or 6 h. This study importantly reported no damage in normal

brain tissue after PDT, mild damage in brain tissue with perifocal edema, and significant tumor damage in the C6 glioma group. Phototoxic damage was observed histologically as either coagulative or hemorrhagic necrosis, sometimes with perilesional pallor. However, in some cases, phototoxic damage was not homogeneously observed throughout the tumors, and residual nests of viable tumor cells were contained within apparently damaged tumor tissue.

Hirschberg et al. reported HGG spheroid cell growth to be more greatly inhibited by repetitive 5-ALA PDT sessions over long intervals (weekly for up to 3 weeks) than by a single-treatment regimen [86]. To test these findings *in vivo*, BT4C HGG tumors were orthotopically implanted in BD-IX rats. Three days after tumor cell implantation, 5-ALA (125 or 250 mg/kg) was administered IP 4 to 5 h prior to PDT. PDT was performed interstitially through the burr hole at the same depth used for tumor cell implantation. Light at 632 nm from a 400 μm flat cut fiber (4.5 – 54 J) was delivered for 10 – 30 min at optical output powers of 7.5 – 30 mW. The rats treated with multiple (i.e. 2 or 3) weekly PDT sessions had a significantly prolonged median survival compared to those treated with a single PDT session.

Most recently, Tetard et al. published efficacy data using a preclinical 5-ALA PDT rat model [87]. Human U87MG GBM cells were orthotopically implanted into athymic fox1 rnu/rnu male rats. Fourteen days post-tumor cell implantation, 5-ALA (100 mg/kg) was administered IP, followed 5 h later by PDT. Light (635 nm) from a diode laser was delivered through a 350 μm flat cut quartz fiber that was intracranially implanted into the tumor under MRI guidance. Overall, 26 J of energy was delivered either in one dose or fractionated (5 J followed by 21 J after a 120 sec break) at a power of either 4.8 mW or 30 mW. The animals treated with interstitial 5-ALA PDT at 30 mW demonstrated signs of elevated intracranial pressure (ICP), which was fatal in approximately 60% of the animals. No fatal or severe adverse effects were observed in the 4.8 mW group. Significant tumor necrosis was induced in both the low and high fluence rate groups, and the results suggested that fractionated PDT was more effective than a single PDT session, though the per-group sample sizes were quite small.

5-ALA Interaction with Other Compounds/Drugs

Some compounds have been identified to increase or decrease the bioavailability of PpIX in brain tumors and therefore possess the potential to respectively improve or impede treatment response to 5-ALA PDT. Phenytoin, a first-generation antiepileptic drug given to brain tumor patients, was found to result in a 55% decrease in PpIX synthesis after administration of 5-ALA in two *in vitro* human U373 MG and U87 MG HGG models [88]. Given this decrease, the authors went on to test the efficacy of 5-ALA PDT to the phenytoin-exposed cells, but the drug was not found to impede the PDT response. The same methods were then used by these authors to investigate any inhibitory effects of levetiracetam, a second-generation antiepileptic drug, but no significant effects on PpIX bioavailability or PDT efficacy were found. Another group reported decreases in PpIX production, albeit increases in PpIX retention, in U87MG cells exposed to dexamethasone and 5-ALA [89], while others showed dexamethasone to decrease blood brain barrier (BBB) permeability in an *in vitro* rat

glioma model [90]. In the latter study, C6, 9L, and T98G HGG cells were incubated with dexamethasone (0.1 μM) for 72 h prior to measuring permeability. Permeability was quantified using the transendothelial flux of radiolabeled sucrose across the *in vitro* endothelial cell monolayer. This group did not perform 5-ALA PDT, but further experiments are warranted to determine if decreased dexamethasone-associated BBB permeability may have an inhibitory effect on 5-ALA PDT. These results suggest that steroid dosages may require careful consideration in patients with HGGs undergoing 5-ALA PDT. A lower steroid dose may be needed to effectively balance the beneficial effect of steroids with the treatment efficacy of 5-ALA PDT.

Iron chelation, vitamin D (calcitriol) supplementation, and ABCG2 inhibition represent several approaches to enhance PpIX accumulation in brain malignancies. In one study, glioma stem cells (GSCs) were incubated with 5-ALA in the presence or absence of deferoxamine, an iron chelator [91]. Deferoxamine was able to increase the low levels of PpIX that were characteristic of GSCs. In a similar study, the efficacy of 5-ALA PDT in combination with administration of the iron chelator 1,2-diethyl-3-hydroxypyridin-4-one hydrochloride (CP94), was investigated. As expected, CP94 enhanced PpIX levels and PDT cytotoxicity in human glioma cells [92]. In another approach, calcitriol was used to selectively increase PpIX accumulation in glioma cells compared to astrocytes [93]. The mechanism for this increase was attributed to the effects of calcitriol on an enzyme in the heme biosynthesis pathway [93]. Finally, the targeting of ATP-binding cassette transporter ABCG2, a well-studied protein that acts to efflux drugs from cells, could also have a role in improving PpIX accumulation and correspondingly PDT efficacy against HGGs [94].

PDT in Combination with Adjuvant Therapies

5-ALA PDT in combination with other therapies has been explored by many groups. Hirschberg et al. reported enhanced tumor cell death following 5-ALA PDT in combination with adjuvant hyperthermia (HT) [21]. In this *in vitro* study, two different HGG spheroid cell lines (human ACBT GBM and rat BT4C HGG cell lines) were incubated in 100 or 500 μg of 5-ALA for approximately 4 h prior to light delivery at 635 nm (fluences of 12 or 25 J/cm^2). HT experiments were performed at temperatures ranging from 37 to 49°C for at least 40 min. The cells in the combination therapy group underwent PDT concurrently with HT within an incubator. Afterwards, individual spheroids were placed in wells and monitored for growth. As individual therapies, hyperthermia at temperatures up to 46°C produced little effect on cell survival, and similarly, PDT alone (37°C) was also minimally effective. In contrast, the combination therapy had a significantly greater inhibitory effect on cell survival that increased as a function of both higher temperature (below 49°C) and greater light fluence. In another approach, hypothermia has also been combined with 5-ALA PDT in a rat RG2 glioma model as a means to increase PpIX accumulation in tumor while protecting normal brain tissue [95]. Four hours prior to PDT delivery, 5-ALA was delivered by IP injection. PDT was performed for 1333 sec (24 J, 635 nm light) via an isotropic emitter that was inserted 1 mm below the dura. Mild hypothermia was performed for approximately 3 hours at 32 – 34°C. Hypothermia increased PpIX fluorescence in tumors by five-fold, spared normal brain tissue, and increased median post-PDT survival time to 14 days compared to 9 days for normothermia PDT.

5-ALA PDT has also been shown to increase the permeability of the BBB to high molecular weight substances such as dextran and Evans Blue dye [96]. In this study, healthy mice underwent a single session of PDT 30 min after IV administration of 5-ALA (20 mg/kg). Fluence rates of 40 – 100 mW/cm^2 were administered to achieve light doses of 10 – 40 J/cm^2 (250 – 400 sec). Spectrofluorometric assay of Evans Blue dye extravasation, confocal microscopy of dextran extravasation, and histological analysis of the BBB permeability to solutes were performed to assess alteration in BBB permeability. In conjunction with a 5-ALA dose of 20 mg/kg, a light dose of 15 J/cm^2 was optimal for BBB opening to high weight molecular substances; however, higher 5-ALA doses were associated with damage to the BBB and brain tissue. These findings suggest the possibility for 5-ALA PDT-induced enhancement of chemotherapy delivery to HGGs, particularly to residual tumor cells.

5-ALA PDT in Clinical Trials

Early phase clinical trials have been conducted using PDT with photosensitizers other than 5-ALA in patients with GBM [37, 97-100]. However, only a few large Phase III studies have been reported [37, 101]. One group combined FGS and post-operative PDT in a Phase III trial [101]. In this clinical study, Photofrin and 5-ALA PDT (630 nm) was performed with an implanted catheter in patients with primary GBM on the day of FGS once the patient recovered from surgery. Photofrin PDT was then performed at 24-hour intervals for a total of 5 PDT sessions. Patients in the control group underwent conventional surgical resection. The treatment and control groups each received fractionated RT after surgery, as part of the standard of care. Delayed mean tumor progression (8.6 vs. 4.8 months) and increased mean survival (52.8 vs. 24.6 weeks) was observed in GBM patients treated with FGS followed by PDT compared to patients who did not receive PDT after conventional surgery. Another group planned a Phase III trial in patients with newly diagnosed or recurrent supratentorial malignant gliomas using Photofrin as a photosensitizer [37]. Intraoperative PDT of the residual tumor bed with low to moderate light doses was performed immediately after surgical resection using an intracavitary balloon. This study was not completed as the authors reported failure to meet the enrollment goals [37].

A limited number of clinical trials using 5-ALA PDT as a treatment modality have been conducted in patients with HGGs (detailed in Table 1). In a Phase I pilot study, 10 patients with non-operable recurrent malignant gliomas underwent interstitial 5-ALA PDT [46]. In this study, 3-dimensional treatment planning software was used to calculate the treatment volume and position of fiber-based cylindrical light diffusers. One hour prior to surgery, 5-ALA (20 mg/kg) was dissolved in 100 mL of water and administered orally to each patient. Up to 6 cylindrical light diffusers (20 or 30 mm) were then placed intraoperatively to provide for illumination of the entire tumor volume. Light was delivered at a power of 200 mW/cm per diffuser length for a total illumination time of 1 hour (720 J/cm) to tumors between 2.1 and 10.2 cm^3 in volume. The mean applied light fluence was 7,212 J, with a mean total volume fluence of 1,405 J/cm^3. No adverse effects of 5-ALA PDT were observed in this study, with asymptomatic perilesional edema resolving over 3 months after PDT. The authors reported a 1-year survival of 60% and a median survival of 15 months versus an expected survival of 6 – 8 months for recurrent malignant gliomas.

More recently, Johansson et al. completed a pilot study for safety and feasibility of interstitial 5-ALA PDT in 5 patients with nonresectable GBM tumors [76]. Prior to 635 nm laser irradiation, tumor biopsies were collected for PpIX quantification and correlated with intra-operative fluorescence measurements. The authors found that the patients with high PpIX levels and complete photobleaching after PDT had favorable long-term outcomes. These authors have presented an update to this research comparing standard adjuvant treatment and interstitial 5-ALA PDT in 15 patients with small and unresectable GBM tumors to a group of patients with complete resection and standard adjuvant treatment [102]. The PDT group had significantly longer progression free survival and 3-year survival compared to the surgical resection group.

In an ongoing Phase I pilot study in France, 10 patients with GBM amenable to complete surgical removal will receive 5-ALA FGS and intraoperative PDT in combination with current standard of care [4] postoperatively [43, 103]. 5-ALA will be administered to patients 4 h prior to surgical resection of the tumor. After intraoperative MRI to assess the extent of surgical resection, PDT will be delivered as 5 fractions of 5 J/cm^2 separated by 2 min breaks in between fractions. Endpoints include toxicity assessment and analyses of survival and quality of life; immunological responses and biomarkers will be assessed [103, 104].

Altogether, the reports published by the above groups demonstrate the safety and potential efficacy of 5-ALA PDT as a treatment modality in patients with HGGs. However, the majority of clinical trials using PDT in the treatment of HGGs are uncontrolled phase I and II studies using a small number of patients which makes it difficult to generalize any survival or tumor progression benefits to the overall population of patients diagnosed with HGGs. Furthermore, the heterogeneity of these clinical studies in terms of design and execution methods, adjuvant therapies used, tumor subtypes, and diagnosis (primary vs recurrent tumors) complicate the assessment of PDT efficacy. Moreover, different approaches of PDT clinical use in terms of dose of photosensitizer and light, methods of light application, and use of techniques in addition to PDT, such as FGS, make it difficult to understand PDT state-of-the-art and predict the future direction of PDT in the treatment of HGGs. Finally, PDT efficacy in HGG therapy has to be compared with the current standard-of-care. For PDT to be a mainstream approach for the treatment of patients with HGGs, an additional patient benefit has to be demonstrated when used in combination with the standard of care. The role of PDT in the treatment of HGGs will be more clearly determined when PDT reaches the point of large, multi-center, randomized controlled Phase III trials.

5-ALA PDT Limitations and Advantages

Compared to Photofrin PDT of HGGs, 5-ALA has fewer side effects [46]. Photofrin has been reported to increase the risk of neurological injury and permanent deficits at a total applied light dose above 4,000 J using diffusion tip fibers [105]. It has also been associated with normal brain tissue damage due to vascular localization of the compound [86]. PDT with 5-ALA, delivered with cylindrical light diffusers and fluences in the range of 4,320 to 11,520 J, can be safely applied in patients [46, 105]. 5-ALA is not cytotoxic when applied systemically and does not appear to significantly redistribute by peritumoral edema bulk

flow [46]. 5-ALA has been associated with minor adverse reactions, including mild elevation of liver enzymes, brief skin photosensitivity, nausea, vomiting, and hypotension [85, 106, 107]. Reports of elevated creatinine levels after 5-ALA administration are likely due to interference of 5-ALA with the Jaffe method of screening; non-Jaffe enzymatic methods of assessing serum creatinine did not identify similar increases [108]. In a study in which 42 patients were given oral 5-ALA (30 mg/kg or 60 mg/kg) prior to abdominal surgery, 15% and 5% of the patients experienced nausea and vomiting, respectively, with increased incidence of both side effects at the higher 5-ALA concentrations [109]. However, no phototoxicity, objective neurologic dysfunction, visual symptoms, hypertension, tachycardia, pain, or hematological abnormality was observed after 5-ALA administration, even at 60 mg/kg. In one case report, 5-ALA was reported to be the potential cause of intraoperative lactic acidosis, in which the patient showed no adverse effects after 24 h [106].

Notwithstanding precautions to be taken in the use of 5-ALA, a major advantage of this photosensitizing agent is its suitability for both FGS and PDT. Recent FDA approval of 5-ALA for FGS has expedited the use of 5-ALA in neurosurgery, thus the feasibility to perform PDT with the same dose of administered drug is not to be underestimated. The multiple mechanisms of action by PDT – direct cytotoxicity to tumor cells, damage of tumor vasculature, and/or stimulation of anti-tumor immunity – are complementary and thus, through their collective effects, can best provide for tumor clearance and control. With regard to these mechanisms, immune effects are expected to be particularly important in the clearance of brain-infiltrating tumor cells, which often accumulate less photosensitizer and may sit at the margins of light penetration [79]. Of further importance in 5-ALA PDT of HGGs, higher light doses may be used to increase the depth of PDT damage. Light dose can be escalated due to the limited accumulation of PpIX in normal brain tissue, coupled with its rapid destruction (photobleaching) upon illumination. This leads to the beneficial situation in which normal brain tissue is protected from the detrimental effects of high light dose that can serve to increase the extent and depth of PDT damage to malignant brain tissue [78].

The aforementioned advantages of 5-ALA as a photosensitizer indicate this compound may demonstrate enhanced therapeutic efficacy when used for PDT in clinical trials for patients with HGGs.

Future Directions

Despite limited data with the use of 5-ALA PDT for HGG therapy, the existing results have been promising. Approval of 5-ALA by the FDA as an intraoperative optical imaging agent for FGS permits future use of 5-ALA alone as an intraoperative surgical aid and 5-ALA PDT as either an intraoperative or postoperative treatment modality. However, a better understanding of the mechanism of action, efficacy, and adverse effects of 5-ALA PDT specific to treatment of HGG is needed. Additionally, the survival benefit of this treatment modality in combination with the current standard of care must be determined by conducting large randomized multicentered Phase III studies in the future. The ideal cancer therapy not only destroys the primary tumor but also stimulates the immune system to recognize and destroy any remaining tumor cells. Therefore, a greater understanding of PDT's

immunomodulatory effects, particularly for HGGs, is necessary [30, 31]. Collectively, this knowledge may inform the future use of 5-ALA PDT in combination with other adjuvant therapies as part of the treatment regimen for patients with HGGs.

Conclusion

5-ALA PDT utilizes the photosensitizing porphyrin precursor 5-ALA to generate ROS that are cytotoxic to brain tumor cells. As has been learned from 5-ALA FGS studies, 5-ALA is highly specific for HGGs, which reduces the likelihood of damage to the surrounding brain with PDT. Compared to other photosensitizing agents, 5-ALA has not been shown to cause severe side effects. 5-ALA PDT has been reported as an immunopotentiator in pre-clinical models, yet this effect remains to be demonstrated in Phase III randomized control trials in patients with HGGs. Newer-generation photosensitizers may eventually replace 5-ALA and further improve PDT efficacy.

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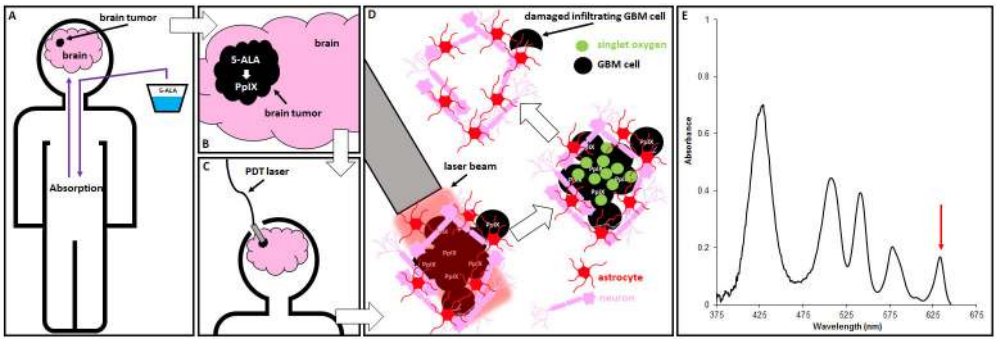

Figure 1.

(A) 5-ALA is administered orally three hours prior to PDT. (B) 5-ALA is converted to PpIX intratumorally. (C) The PDT laser is positioned appropriately during surgery. (D) Upon exposure to an activating wavelength of light, PpIX converts oxygen into singlet oxygen species (~microseconds), resulting in tumor bulk death (~days). Infiltrating tumor cells that uptake 5-ALA, but are not within the region of the laser's effect, may still be damaged after 5-ALA PDT. (E) Absorption spectrum of PpIX, demonstrating its multiple absorption peaks over the range of wavelengths that make up visible light. PpIX (0.06 mg/mL) was dissolved in 50% Solvable/50% deionized water. Absorbance was measured on a spectrophotometer. The red arrow identifies the peak at ~635nm that is used for excitation of PpIX in applications of 5-ALA PDT.

Table 1.

Summary of 5-ALA PDT Clinical Trials for HGG

Study	Patients	ALA Dose/ Drug-Light Interval	Light Delivery	Post-PDT Care/Evaluation	Observations
Beck et al. 2007	10 unresectable recurrent malignant glioma 3 cm max diameter	20 mg/kg ALA 1 h pre-surgery	Interstitial PDT 20 or 30 mm diffusers 200 mW/cm 4,320-11,520 J 633 nm	Evaluation at day 1, 1 month, 3 month intervals with MRI	No perioperative morbidity Asymptomatic edema resolving within 3 months 60% 1 year survival 15 month median survival
Johansson et al. 2013	5 unresectable recurrent malignant glioma 3 cm max diameter	20-30 mg/kg ALA 5-8 h pre-irradiation	Interstitial PDT 20 or 30 mm diffusers 150-200 mW/cm 5,760-12,960 J 635 nm	Evaluation at day 1, 1 month, 3 month intervals with MRI	No morbidity reported No progression in 3/5 patients at 29, 30, and 36 months
Schwartz et al. 2015 (abstract)	15 unresectable de-novo GBM < 4 cm diameter	20-30 mg/kg ALA	Interstitial PDT Median 12,960 J 633 nm	Standard of care after PDT (e.g., radiotherapy plus temozolomide) Median 34 month follow-up	Transient morbidity in 7/15 patients 16 month median PFS * 56% 3-year survival
INDYGO Reyns 2017 (clinicaltrials.gov) Vermandel et al. 2017 (abstract) Dupont et al. 2018	10 resectable newly-diagnosed GBM	20 mg/kg ALA 4 h pre-surgery	PDT of resection cavity after FGR ** 5 fractions of 5 J/cm^2 2 min “light-off” between fractions 635 nm	Standard of care after PDT (e.g., radiotherapy plus temozolomide) Planned MRI evaluations Quality of life assessments Blood for immune assays	In recruitment

* PFS, progression free survival

** FGR, fluorescence-guided resection