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Abstract. Photodynamic therapy (PDT) effect is a promising adjuvant modality for diagnosis and treatment of brain cancer. It is of importance that the bright fluorescence of most photosensitizers provides visualization of brain tumors. This is successfully used for fluorescence-guided tumor resection according to the principle “to see and to treat.” Non-oncologic application of PDT effect for induction of photothrombotic infarct of the brain tissue is a well-controlled and reproducible stroke model, in which a local brain lesion is produced in the predetermined brain area. Since normal neurons and glial cells may also be damaged by PDT and this can lead to unwanted neurological consequences, PDT effects on normal neurons and glial cells should be comprehensively studied. We overviewed the current literature data on the PDT effect on a range of signaling and epigenetic proteins that control various cell functions, survival, necrosis, and apoptosis. We hypothesize that using cell-specific inhibitors or activators of some signaling proteins, one can selectively protect normal neurons and glia, and simultaneously exacerbate photodynamic damage of malignant gliomas. © 2015 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.20.6.061108]

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1 Photodynamic Effect

Photodynamic effect, an injury of stained cells under light exposure in the presence of oxygen, was discovered by Raab and von Tappeiner in 1900. In 1903, von Tappeiner and Jesionek cured human skin cancer stained by eosin and exposed to sunlight. These experiments were, however, discontinued because of the weak therapeutic effect, toxicity, and carcinogenicity of eosin.¹ The widespread application of photodynamic therapy (PDT) in oncology was initiated 75 years later, when Dougherty et al. reported the complete or partial remission of 111 from 113 cutaneous and subcutaneous tumors and metastases in 25 patients photosensitized with hematoporphyrin derivative (HpD).^{2,3}

In PDT, photon absorption induces photoexcitation of dye molecules. The lifetime of the excited singlet state is $\sim 10^{-8}$ to 10^{-9} s. After that, the excited dye molecule emits photons and returns to the ground state. However, it can turn into the long-lived triplet state with a much longer lifetime (10^{-4} to 10^{+2} s). During this time, the excited photosensitizer (PS) molecule can participate in redox reactions with electron or proton transfer and formation of intermediate radical products, which then react with oxygen (type I reactions). Alternatively, it first reacts with oxygen and converts it into the highly reactive singlet form, $^1\text{O}_2$, (type II reactions).^{4,5} Dougherty and coworkers identified $^1\text{O}_2$ as the main cytotoxic agent in the photodynamic damage of a tumor.⁶ Type II reactions dominate in the PDT effects of most PSs due to greater oxidizing ability and higher $^1\text{O}_2$ diffusion coefficient.⁵ Singlet oxygen intensely oxidizes cellular proteins and membrane lipids.⁷ It is of importance that in cells, the diffusion path of $^1\text{O}_2$ is <10 to 20 nm.^{8,9} Therefore, it

oxidizes only cellular structures in the nearest vicinity of PS molecules. $^1\text{O}_2$ and other reactive oxygen species ($\text{O}_2^\bullet$, $\text{OH}^\bullet$, etc.) oxidize unsaturated fatty acids in biomembranes and convert them into lipid radicals ($\text{L}^\bullet$), alkoxy radicals ($\text{LO}^\bullet$), peroxy radicals ($\text{LOO}^\bullet$), or hydroperoxides (LOOH). They initiate chain lipid peroxidation leading to dysfunction of biomembranes, oxidative stress, and finally to cell death.^{5,10-12}

2 Photodynamic Therapy

PDT is a binary effect. Its components, PS and light, are non-toxic individually, and only their combination, i.e., PDT, kills cells. Its advantages include local, selective, and contactless action, good wound healing without large scar formation, the possibility to repeat the treatment and to combine it with other treatment modalities. PDT procedure consists of a range of stages.^{4,12}

1. PS delivery and selective tumor staining: PS pharmacokinetics depends on tissue features, such as blood supply, and on dye properties. Hydrophilic PSs localize mainly to blood vessels, whereas hydrophobic dyes penetrate into tumor cells. After administration, PS selectively accumulates in a tumor, where its level may be 10 to 30 times higher than in the surrounding healthy tissue. Afterward, it is gradually eliminated from the body. This allows selective tumor destruction. Hydrophilic PSs first are adsorbed on the cell surface and then penetrate into endosomes and lysosomes by pinocytosis. Hydrophobic PSs localize inside the plasma membrane and membranes of intracellular organelles. They are more effective, but when administered intravenously, they can aggregate and precipitate. The best

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results were obtained with amphiphilic PSs that contain both hydrophobic and hydrophilic parts.

2. Photophysical processes: Powerful lamps, lasers, or light-emitting diode (LED) arrays emitting red or near-infrared light (600 to 800 nm) are used in PDT. In this spectral region, light absorption of blood hemoglobin and muscle myoglobin is weakest, and light penetrates through skin up to 1 cm. Infrared light (>800 nm) is inefficient due to its low ability of $^1\text{O}_2$ generation.^{4,5} Oncologic applications of PDT are limited to superficial cancers, like skin, head, and neck, or breast tumors, which can be irradiated directly, or to abdominal tumors, such as mouth, esophagus, stomach, colon, lung, cervical, prostate, and bladder cancers, to which light may be delivered by means of optical fibers.
3. Secondary photochemical processes leading to cell death and tumor destruction: As a result of $^1\text{O}_2$ -mediated lipid peroxidation and oxidation of proteins, the cytotoxic cascades leading to oxidative stress, necrosis, or apoptosis are developed in the photosensitized cells. These processes are regulated by the intracellular signal transduction reactions and may be either facilitated or suppressed by pharmacological modulation of signaling proteins. Cell debris is absorbed, digested, and excreted by neighboring cells or blood leukocytes.
4. Wound cleansing and healing: Intense PDT induces massive necrosis with formation of scar tissue. Relatively weak PDT mainly causes apoptosis. In the case of apoptosis, a wound heals rapidly and a scar is not formed.¹³
5. PS excretion from the body: After intravenous PS administration, the patient can acquire cutaneous photosensitivity for a long time, up to several months that limits being outdoors and reduces the working capacity and the quality of life. So PS must be rapidly eliminated from the organism.

The optimal PS should have the following properties: non-toxicity, high absorption of red light (600 to 800 nm), high quantum yields of triplet states and $^1\text{O}_2$ generation, fast and selective accumulation in tumors, rapid elimination from the body after the treatment, and low cost.^{12,14} Some of these requirements are contradictory, so the design of new drugs has to be a compromise.

Many PSs are currently synthesized and tested: porphyrins, chlorines, phthalocyanines, etc. Some promising PSs have already passed the II and/or III stage of clinical trials. HpD (Photofrin), 5-aminolevulinic acid (ALA), a natural precursor of protoporphyrin IX (PpIX), ALA methyl derivative (Metvix), m-tetrahydroxyphenyl chlorine (mTHPC or Foscan), hypericin, benzoporphyrin derivative BPD-MA (verteporfin or vizudin) are approved for treatment of various cancer types in different countries.^{1,15–17}

3 PDT Applications in Neurooncology

Brain tumors are responsible for 2% of all cancers. High-grade gliomas, such as glioblastoma multiform (GBM) and anaplastic astrocytoma, are the most dangerous brain tumors (~70 and

11%, respectively). The average lifespan of patients with glioblastoma is less than one year. GBMs are resistant to chemotherapy and radiotherapy. Surgery is mostly inefficient because of the inability to perform wide tumor resection without injury of surrounding normal nervous tissue and due to the high infiltration of glioma into the normal tissues. Small tumor twigs, which were not noticed by a neurosurgeon and remained in the brain tissue, can stimulate tumor recurrence beyond a resection margin. Current treatment regimes, such as surgery, radiotherapy, and chemotherapy, can prolong the median patient survival to 15 months. Cases of a complete cure of brain tumors using different treatment methods are rare.^{18–23}

ALA-produced PpIX, Photofrin, Foscan, or boronated porphyrin (BOPP) were used in PDT of GBM. Under light exposure (635 nm, 100 J/cm² for ALA-PpIX; 630 nm, 60 to 260 J/cm² for Photofrin; 652 nm, 20 J/cm² for Foscan, and 630 nm, 25 to 100 J/cm² for BOPP), photogenerated $^1\text{O}_2$ induced oxidative stress and killed GBM cells.^{18,19,22–26} Argon-dye laser, gold vapor laser, or xenon arc lamps with adequate filters have previously been used as light sources. Currently, LEDs and diode lasers are preferable because of their reliability, compact size, and low cost.²³ Optical fibers are often used for light delivery. However, the irregular shape and large volume of tumors create significant problems for light delivery, distribution, and dosimetry. To achieve the homogeneous light diffusion in the tumor cavity, an inflatable balloon filled with a light-diffusing lipid solution and inserted into the tumor cavity is used. This is important for irradiation of tumor margins, where 90% of GBM recurrence occurs.^{23,27,28} Hirschberg et al. designed an implantable light applicator for repetitive PDT.²⁹

Selective accumulation of PSs in the GBM tissue (ranging from 2:1 with HpD to 400:1 with BOPP as comparing with normal brain cells) and good fluorescence provide tumor visualization. Fluorescence-guided surgical resection (FGR) is performed according to the slogan “to see and to treat.”^{18,19,21,23,27,29} This substantially improves glioma treatment.^{19,27,30–33} Good results have been achieved with the neurosurgical microscope equipped with the 375 to 440 nm filter for fluorescence excitation of ALA-produced PpIX, and the long-pass filter (>455 nm) for visualization of PpIX fluorescence (at 635 nm) in the tumor.²⁷ Similarly, Foscan-mediated FGR allowed visualization of thin glioblastoma twigs, which, if undetected, can induce tumor relapse. The following tumor excision was successful in 60 to 70% of patients.^{23,30,33} After FGR, the operation field and tumor margin can be additionally subjected to photodynamic treatment mediated with ALA/PpIX^{18,19,27,32,34} or Foscan.³⁰ So these PSs work twice: first, for tumor detection and monitoring of the surgical operation, and second, for photodynamic treatment. This provides more reliable destruction of the remaining cancer cells.²⁴

PDT is particularly effective in combination with other treatment modalities. The best effect is achieved with the combination FDR+PDT+radiotherapy+chemotherapy.^{19,23,24,26,34,35} According to a nonrandomized comparison of different adjuvant therapies for GBM, PDT significantly increased the median 12- and 24-month survival of patients subjected to surgical resection and external beam radiation therapy from 35 and 8 to 80 and 50%, respectively.¹⁸ Stilly and Kaye have reported that 28% of patients with primary GBM survived >24 months, and 22% of patients >60 months. The treatment of recurrent GBM patients was more promising: 41% survival beyond 24 months.²² According to Kostron, the median survival of PDT-treated patients with

primary GBM was 22 months and for recurrent GBM was 9 months as compared to the standard conventional treatment (15 and 3 months, respectively). FGR demonstrated very positive results.²³ The combination of photodynamic diagnosis/FGR and intraoperative PDT (“to see and to treat”) offers a promising approach to the treatment of malignant brain tumors.

In the intact brain, neurons are protected by the blood-brain barrier from diverse chemical agents, including PSs. However, in brain tumors, the blood-brain barrier is partly disrupted, and they accumulate more ALA-induced PpIX than normal tissue.^{32,34,36} ALA-PDT disrupts blood vessels and causes edema that provides PS penetration, exacerbates injury of the nervous tissue, and kills neurons.^{34,36} As shown *in vitro*, ALA-PDT induces massive apoptosis of glioma cells via the proapoptotic mitochondrial pathway.^{37,38} However, some authors demonstrated ALA-PDT-mediated necrosis in various glioma cell lines,³⁹ spheroids,³⁹ and glioblastoma tissues.⁴⁰

The dynamics of early molecular events in the cultured glioblastoma D54Mg cells caused by weak ALA-PDT that killed no more than 5% of the cells was characterized in a recent work.⁴¹ Using the antibody microarrays that reveal the expression of 224 proteins involved in signal transduction, the authors observed phosphorylation of protein kinase Raf, adhesion-related kinases FAK and Pyk2, and microtubule-associated protein tau 0.5 to 1 h after PDT. Protein kinase C γ and microtubule-associated protein MAP-1B were overexpressed. At the same time, the components of the actin cytoskeleton scaffold, such as dystrophin, calponin, and vinculin, microtubule-associated proteins MAP2 and CNP, components of the intermediate cytoskeleton cytokeratins 4 and 7 were downregulated. These processes are associated with the changes in cell shape and adhesion. Downregulation of cyclins A, D1, and D3, c-Myc, checkpoint proteins chk1/2 and upregulation of Smad4 could be associated with the cell cycle arrest. Overexpression of Bcl-xL and downregulation of caspase 9 demonstrated the antiapoptotic response. At 5.5 h after PDT, the levels of protein kinase C γ and β -synuclein as well as phosphorylation of Raf, FAK, Pyk2, and tau were still increased.⁴¹ Nuclear factor NF- κ B is a key regulator of various physiological processes, including cell growth and apoptosis. Its inhibition enhanced the death of human glioblastoma cells induced by ALA-PDT. In these cells, PDT induced mainly necrosis, and NF- κ B played the antinecrotic role. Inhibition of NF- κ B reduced the level of apoptosis, i.e., it rendered glioblastoma cells more photosensitive.⁴²

4 Photothrombotic Stroke

The photothrombotic stroke is an example of the nononcologic application of PDT. Ischemic stroke, which accounts for ~80% of strokes, is one of the major factors of human disability and death. Acute focal ischemia induces cell death not only in the infarction core but also in the surrounding tissue (penumbra). Oxygen and glucose deficit in the infarction locus very quickly, for a few minutes, leads to ATP depletion, generation of reactive oxygen species, injury of cellular membranes, loss of ionic gradients, depolarization, excitotoxicity, cell death, and tissue edema. These injurious processes spread to the surrounding tissue. The tissue damage in the penumbra develops slower, during several hours, and this therapeutical window provides time for the protection of cells in this zone and the decrease of deleterious neurological consequences. However, the current neuroprotective drugs are not sufficiently effective. Therefore, the comprehensive study of neurodegeneration and neuroprotection

in the penumbra is necessary for the development of novel approaches for treatment of stroke consequences.^{43,44} Current models of brain ischemic stroke such as ligation of the middle cerebral artery, or its occlusion by a nylon thread, or injection of thrombogenic factors do not provide localized and predictable brain infarction.

In 1985, Watson and coworkers had suggested the use of the photodynamic effect of hydrophilic PS Bengal Rose for induction of focal photothrombotic infarction (PTI) in the rat cerebral cortex.⁴⁵ After intravenous administration, Bengal Rose does not cross the blood-brain barrier and penetrates into neuronal cells. It accumulates in the brain microvessels. The following laser irradiation induces local oxidative damage of endothelial membranes, platelet aggregation, and occlusion of microvessels followed by local blood flow interruption. PTI is a noninvasive stroke model. It does not require craniotomy and mechanical blood vessel manipulations, such as ligation or insertion of nylon filament, which carry the risk of local brain trauma. The lesion location, size, and degree are predictable and well reproducible. They are easily controlled by the irradiation intensity and duration, beam position, and dye concentration.^{45–48} PTI is characterized by a low animal mortality (<10%) and prolonged sensorimotor impairment.^{49–51} The 2-laser system, in which Bengal Rose-mediated photothrombosis was induced by 568-nm krypton laser irradiation and 355-nm UV light of YAG laser induced reperfusion, provides a less invasive and reproducible focal ischemia.⁵²

The disadvantages of this PTI model are that the light scattering by the skull is rather small, the lesion edges are sharply demarcated, and the penumbra is practically absent. In order to study the neurodegeneration and neuroprotection processes in penumbra, a photothrombotic ring model of rat stroke was developed. In this case, the argon laser beam (514.5 nm, PS erythrosin B) was configured as a 5-mm-diameter ring, and light exposure was decreased. The ischemic penumbra reproducibly proceeded toward the ring center for an extended time period.⁵³ The local cerebral blood flow (CBF) inside the photothrombotic ring declined promptly after irradiation. The central region exhibited morphological alterations: at 4 h after PTI, some neurons appeared swollen, at 48 h, the majority of neurons were severely swollen, eosinophilic, and pyknotic, whereas at seven days poststroke, the tissue morphology became partly normalized like in the cortical penumbra in other models of cerebral ischemia.⁵⁴

Hemodynamic and vascular changes are of great importance for ischemic stroke development. Novel optical methods have been developed recently for monitoring CBF changes in the animal brain under ischemic stroke. Parthasarathy et al.⁵⁵ developed the multiexposure speckle imaging technique to monitor CBF through the thin mouse skull after photothrombotic occlusion of the middle cerebral artery. In this method, 532-nm laser induced photothrombosis and a 660-nm diode laser was used for speckle contrast imaging. This technique provided an accurate estimation of CBF changes *in vivo* with high spatial and temporal resolution despite the presence of static light scattering elements, such as skull bones.⁵⁵ Using a high-resolution and high-speed CCD camera and automatic parabolic curve fitting, Liu et al.⁵⁶ developed a microscopic laser speckle imaging system for real-time monitoring of CBF changes in cortical vessels induced by photothrombotic stroke. The authors revealed the details of vascular disturbances and the stages of blood coagulation after photothrombotic stroke. They also obtained the

information on additional parameters, such as blood vessel diameter, centerline velocity, and standard deviations of CBF in various areas during thrombus formation with high spatial and temporal resolution. This stroke model showed that the processes of vascular occlusion in main and branch vessels consist of a number of stages with different vascular mechanics and hemodynamics.⁵⁶ The photoinduced damage of the endothelium and following disturbance of the blood-brain barrier leads to tissue edema and to penetration of PS into glia and neurons that contributes to the direct photodynamic injury of these cells.^{36,57}

Photothrombotic stroke model was used in studies of consequences of localized brain infarction. Vandeputte et al.⁵⁸ have demonstrated neurogenesis in the adult mammalian brain following stroke. In order to monitor the fate of the endogenous neural stem cells (eNSCs) after PTI, the authors used the transgenic mice that express firefly luciferase in these cells. An increased bioluminescence signal was observed in the infarct region. In the peri-infarct area (penumbra), the labeled eNSC originating from the subventricular zone proliferated, migrated toward the infarct region, and differentiated into both astrocytes (36%) and neurons (21%).⁵⁸

A rat photothrombotic brain ischemia model was also used to test new antistroke drugs. The neuroprotective effects of diazepam, a blocker of calcium channels,⁵⁹ memantine, the antagonist of N-methyl-D-aspartate glutamate receptor,⁶⁰ leptin, an AMP kinase inhibitor,⁶¹ melatonin that reduces the elevated level of matrix metalloproteinase 9, which disrupts blood-brain barrier,⁶² brain-derived neurotrophic factor,⁶³ and other compounds have been reported.

5 PDT-Induced Effect on Normal Nervous Tissue

Not only brain tumor cells, but also nearby normal neurons and glial cells are injured during PDT. This can induce harmful side effects and neurological disorders. Furthermore, peripheral nerves are inevitably damaged during photodynamic treatment of any tumors. Selective destruction of a brain tumor and simultaneous protection of adjacent normal neurons and glial cells is eligible. Therefore, the detailed study of the mechanisms that regulate the survival and death of photosensitized glioma cells, neurons, and glial cells is needed.

The estimation of the threshold for PDT-induced tissue necrosis in the normal rat brain photosensitized with Photofrin⁶⁴ or aluminum phthalocyanine tetrasulfonate⁶⁵ showed very high vulnerability of the nervous tissue to photodynamic damage. Comparative studies of photodynamic effects of various derivatives of porphyrins,⁶⁶ chlorines,⁶⁷ aluminum, or zinc phthalocyanines⁶⁸ showed their capability to inactivate crayfish mechanoreceptor neurons at nanomolar concentrations. Chlorine derivatives mTHPC and Radachlorine (a mixture of chlorine e6, chlorine p6, and purpurin 5) were effective at subnanomolar concentrations.^{68,69}

Photofrin II-mediated PDT has been shown to induce vascular damage, edema, and necrosis in the rat cerebral cortex. These alterations were observed as early as 4 h after PDT and reached a maximum at 24 h postirradiation.⁷⁰ Damage of the rat brain depended on the light dose: the small dose (1.5 J cm^{-2}) induced limited neuronal injury and neuropil vacuolation similar to the early ischemic lesion. The higher energy levels (35 to 140 J cm^{-2}) caused massive necrosis such as in the case of arterial occlusion.⁷¹ The morphological changes spreading from the cortical surface to the deepest layer involved first astrocytes

(1 h), then endothelial cells, and, eventually, neurons. Thrombi were appeared in the microvessels after 18 h. Coagulation necrosis within the photosensitized area occurred only after 48 h.⁷² Rare apoptotic cells were observed around the necrosis region. PDT-induced necrosis resulted from direct oxidative cell injury, whereas apoptosis was rather associated with secondary effects, such as vascular damage, edema, and hypoxia.⁷³

Ultrastructural changes in the rat cerebral cortex photosensitized with Photofrin II included cisternal swelling of both endoplasmic reticulum (ER) and Golgi apparatus (GA) in the superficial cortical neurons. After light exposure, these changes propagated to the cortex bottom. In the next 18 h, the morphological features of the lethal injury of neurons and astrocytes included electron-dense deposits within swollen mitochondria and fragmentation of the nuclear and cytoplasmic membranes. These alterations progressed to coagulation necrosis during 48 h.⁷⁴

In the mammalian brain, numerous neurons are interconnected with thousands of other neurons. The number of glial cells is much higher than the number of neurons. It is very difficult to identify brain neuronal and glial cells and study the identified neurons and glial cells. It is, therefore, reasonable to study the simpler nervous systems of invertebrates. The crayfish abdominal stretch receptor contains a single mechanoreceptor neuron enveloped by glial cells. In this object, neuroglial interactions are clearly identified.⁷⁵ In the stationary state, this neuron fires regularly with a constant rate for several hours. Photosens-mediated PDT induced elimination of neuronal activity, necrosis of neuron and surrounding glial cells, and apoptosis of glia. Short-term (5-min) PDT that slightly changed neuronal activity by 1 to 2 Hz induced swelling of some mitochondria and ER cisterns. 30-min photosensitization that irreversibly abolished neuronal firing destroyed mitochondria, depleted energy sources (glycogen granules), and impaired granular ER, GA, and polysomes involved in protein synthesis and processing. These ultrastructural changes increased 1 h after PDT and led to necrosis. At this time, the segregation of the neuronal cytoplasm by Nissl bodies, which are involved in protein synthesis and transport along neurites, has been lost. The structures involved in the glia-to-neuron interactions (neuronal submembrane cisterns, glial protrusions that being captured form double-membrane vesicles within the neuronal body), intragial transport (tubular lattices), and intraneuronal transport (Nissl bodies, GA, bundles of microtubules) were impaired at the earlier stages of the stretch receptor damage. Although alterations of intracellular organelles were simultaneously initiated in neurons and glia, glial organelles were eliminated faster. Therefore, glial cells were more sensitive to PDT than neurons.^{76,77}

High photosensitivity of the nervous tissue may be associated with very intense oxidative metabolism, high susceptibility to reactive oxygen species, abundance of photosensitive electron-transferring proteins in the numerous mitochondria, initial photodamage to microvascular endothelium, and initial disruption of the blood-brain barrier by relatively small light doses followed by massive PS penetration.

Biochemical changes in the photosensitized brain have not yet been sufficiently studied. Our recent research showed that ALA-PDT killed some but not all of the neuronal cells and caused microvascular alterations in the mouse cerebral cortex. Using proteomic antibody microarrays, we studied the changes in expression of 112 proteins involved in epigenetic regulation

in the mouse cerebral cortex 1 h after ALA-PDT. The observed changes in expression of epigenetic proteins and histone modifications were directed to suppression of transcriptional activity, impairment of DNA repair, stimulation of proliferation, nuclear protein import, and regulation of cell survival and apoptosis. These changes depended on the time interval after PDT. Major alterations observed in the first hour after the treatment resulted in suppression of transcription and DNA repair. At 4 h after PDT, the changes in the expression of proteins involved in the regulation of proliferation or death of cortical cells were most significant.⁷⁸

Using modulators (inhibitors or activators) of diverse signaling proteins, we studied the involvement of various regulatory systems in the survival and death of photosensitized neurons and glial cells in the isolated crayfish stretch receptor. Local irradiation of the body of the mechanoreceptor neuron by the focused laser beam increased the level of apoptosis but not necrosis of the satellite glial cells that was induced by following a weaker PDT. Therefore, this neuron protects surrounding glial cells from photodynamic injury.⁷⁹ One can suggest that the neuron releases some antiapoptotic molecules that protect glial cells. In fact, neurotrophic factors, nerve growth factor (NGF), and glia-derived growth factor but not brain-derived growth factor or ciliary neurotrophic factor protected glial cells (but not neurons) from PDT-induced necrosis and apoptosis.^{80,81} NGF-mediated protection of glial cells from PDT-induced apoptosis was mediated by mitogen-activated protein kinase JNK.⁸⁰ Unlike another mitogen-activated protein, kinase p38 was involved in PDT-induced glial necrosis but not apoptosis. Nitric oxide (NO), another intercellular messenger, could also influence PDT-induced death of neurons and glia. However, the experiments with chemical NO generators (NONOate or sodium nitroprusside) showed that NO played a proapoptotic role in the photosensitized glial cells but protected neurons from PDT-induced necrosis.⁸²

These intercellular messengers initiate diverse intracellular signaling pathways that control life and death of cells.^{12,83–85} One of these pathways is associated with Ca^{2+} . PDT is known to rapidly increase the cytosolic Ca^{2+} level. On the other hand, Ca^{2+} controls numerous intracellular processes, including necrosis and apoptosis. We showed that the mitochondrial permeability transition pores, through which Ca^{2+} may be released into cytosol, phospholipase $\text{C}\gamma$ that stimulates Ca^{2+} release from ER, and protein kinase C are involved in PDT-induced inhibition of neuronal activity. The multiple signal transduction pathways, including calmodulin, calmodulin-dependent kinase II (CaMKII), adenylate cyclase, protein kinase B/Akt, and glycogen synthase kinase- 3β , were involved in the PDT-induced necrosis of neurons. PDT-induced necrosis of glial cells was mediated by calmodulin, CaMKII, p38, protein kinases A, B, C, and G. In contrast, NO-synthase and NO reduced the level of necrosis of neurons and glial cells. At the same time, mitochondrial permeability transition pores and phospholipase $\text{C}\gamma$, which mobilize intracellular Ca^{2+} , NO synthase, NO, protein kinase G, and glycogen synthase kinase- 3β participated in apoptosis of photosensitized glial cells. In contrast, JNK, adenylate cyclase, protein kinases A and C played the antiapoptotic role in glial cells.^{86–88}

Thus, various signaling pathways modulate both survival and death (necrosis and apoptosis) of neurons and glial cells. The signaling processes in neurons and glial cells are different to some extent. This difference is possibly associated with different molecular messengers and their receptors involved in the

inter- and intracellular signaling pathways and with different sets of specific execution proteins in neurons and glia. Their modulation by various inhibitors or activators can modify the efficacy of PDT: protect one cell type and exacerbate the damage of others.

6 Conclusion

PDT effect is a promising adjuvant modality for diagnosis and treatment of brain cancer. It is of importance that the bright fluorescence of most PSs provides visualization of brain tumors that is successfully used for fluorescence-guided tumor resection according to the principle “to see and to treat.” Non-oncologic application of the PDT effect for induction of photothrombotic infarct of the brain tissue is a well-controlled and reproducible stroke model, in which the local brain lesion is produced in the predetermined brain area. Since normal neurons and glial cells may also be damaged by PDT and this can lead to unwanted neurological consequences, PDT effects on normal neurons and glial cells should be comprehensively studied. We briefly reviewed the current literature data on the role of some signaling and epigenetic proteins, which control various cell functions, survival, necrosis, and apoptosis, in the PDT effect on neurons and glial cells. We hypothesize that by using cell-specific inhibitors or activators of some signaling proteins, one can protect selectively normal neurons and glia, and simultaneously exacerbate the photodynamic damage of malignant gliomas.

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