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## Photodynamic therapy of malignant brain gliomas

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Treatment of malignant gliomas of the brain remains a serious problem on a global scale, despite intensive research into the causes and mechanisms of their progression. When using traditional surgical approaches and imaging methods tumor cell infiltrates may be overlooked, as a result of which, malignant gliomas relapse often occurs near the marginal region of the surgical cavity. A method that allows visual identification of tumor tissue and at the same time provides an opportunity to selectively destroy it is photodynamic therapy (PDT) – a two-stage treatment that includes the introduction (intravenous, intraperitoneal, local or oral) of a light-sensitive chemical agent (photosensitizer (PS)) followed by its activation at a certain wavelength of light.

The principle of PDT is based on the cytotoxic effects caused by PS, which selectively accumulates in malignant tumor cells and is activated by light rays of the appropriate wavelength, generating singlet oxygen and free radicals, which trigger photochemical reactions in tumor cells with subsequent destruction of protein structures. Tumor tissue has a higher affinity for PSs. PSs are divided into 1st, 2nd and 3rd generation molecules. So far, 3rd generation PSs have not yet been approved for clinical use. *In vitro* and *in vivo* experimental studies confirmed the effectiveness of PDT of brain tumors using 2nd generation PSs.

The simultaneous use of surgery under the control of fluorescence and PDT enables both the visualization of tumor cells and their selective destruction. Regardless of PDT, PSs are used for the purpose of auxiliary delineation of tumor borders for maximum tumor removal during fluorescence-guided surgery.

The review examines the development of PDT in a historical aspect, the contribution of domestic scientists, in particular, scientists of the Institute of Neurosurgery named after acad. A. P. Romodanov, National Academy of Medical Sciences of Ukraine to the development of the problem of PDT in neuro-oncology; preclinical studies of PDT and experimental approaches to increase the efficiency of PDT are characterized. Analysis of data from clinical trials confirms that using PDT as an adjunctive treatment of malignant gliomas administered immediately after maximal resection is safe, reduces the risk of recurrence by targeting residual tumor cells in the resection cavity, improves survival and quality of life of patients. The absence of information on the development of resistance to multiple PDT sessions suggests the possibility of repeated treatments of tumor cells not removed during surgery.

**Key words:** laser radiation; photosensitizer; antitumor photodynamic therapy; malignant brain gliomas

Malignant neoplasms are a global medical and social problem with a tendency to a steadily increasing morbidity and mortality rates (primarily due to malignant gliomas (MG)) [1]. Treatment of malignant brain gliomas remains a great challenge on a global scale, despite intensive research into the causes and mechanisms of their progression.

### Etiology and epidemiology of malignant brain gliomas

Gliomas are conditionally divided into two broad groups [2]: circumscribed gliomas, which have relatively

well-defined margins and can be completely removed surgically, and diffuse gliomas, which have no clear borders and cannot be totally removed during surgery.

In 2021, the classification of central nervous system (CNS) tumors was revised taking into account molecular biological markers (5th edition) [3]. According to the new edition, diffuse gliomas in adults are classified into three groups: astrocytomas (IDH mutation, CNS WHO grade 2, 3, 4), oligodendrogliomas (IDH mutation and 1p19q codeletion, CNS WHO grade 2, 3), glioblastomas (IDH-wildtype, CNS WHO grade 4), in children - into low-grade and high-grade with corresponding

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subtypes. Circumscribed astrocytic gliomas include pilocytic astrocytomas, high-grade astrocytomas with piloid features, pleomorphic xanthoastrocytomas, subependymal giant cell astrocytomas, chordoid gliomas, astroblastomas (MN1-altered) [3].

According to CBTRUS statistical report of for 2014–2018 (the previous edition of the CNS tumor classification of 2016 was applied), the average annual incidence rate of malignant brain tumors and other CNS tumors was 7.06 per 100,000 population, the average annual death rate was 4.43 [2, 4]. The most common malignancy among brain tumors and other CNS tumors is glioblastoma (GB) (14.3% of all tumors and 49.1% of malignant brain tumors). The five-year relative survival rate after morphological diagnosis of a malignant brain tumor and other CNS tumors was 66.9%, whereas for non-malignant variants of brain tumors and other CNS tumors it was 92.1%. Diffuse gliomas had a significantly worse prognosis than circumscribed/demarcated gliomas, and differed significantly in the duration of overall and recurrence-free survival of patients depending on the histological variant [2].

The incidence of malignant variants of primary brain tumors in Ukraine, according to the latest data of the National Cancer Registry (2020), is 5.0 per 100,000 population, the mortality rate is 3.5 per 100,000 population [5]. Among primary CNS tumors, malignant forms of brain gliomas (WHO grade 3-4) predominate, most of them are GB (WHO grade 4). The survival analysis of patients with oligoastrocytomas (163 cases for the period from 2005 to 2015) showed that the median overall survival was (49.9±2.4) months and differed depending on the histostructure of the tumor: in patients with predominance of the oligodendroglial pool, the average overall survival of patients who underwent surgery was (100.5±4.6) months, with predominance of the progression of the astrocytic component of the proliferate - (48.2±4.5) months, with approximately equal distribution of the oligodendroglial and astrocytic components - (76.6± 4.9) months [6]. The survival analysis of patients with primary GB in Ukraine (3763 cases for the period from 2008 to 2016) showed that the median overall survival was (12.2±0.2) months, the average overall survival of operated patients was (7.5±0,3) months, when using combined therapy (surgical removal, radiation and chemotherapy) – (16.3±0.5) months ( $p < 0.001$ ) [7].

Consequently, the results of complex treatment of GB remain unsatisfactory due to the invasive nature of their spread and multiresistance to adjuvant treatment methods and require the development of new and improvement of existing methods for the management of this pathology. The modern standard of treatment for GB involves surgical treatment with maximum functionally possible resection radiochemotherapy and adjuvant chemotherapy [8]. However, despite advances in neurosurgery, chemotherapy, and radiotherapy, GB remains one of the most treatment-resistant CNS malignant neoplasms and the tumor inevitably progresses. Most cases of continued growth/recurrence occur in or near the resection area, namely the area that received the highest dose of postoperative radiation [9]. Many new therapies are designed to combat these local recurrences by administering therapeutic agents directly into or near the tumor bed.

The resistance of GB in response to curative measures is widely known and can be explained by several features: GB is a heterogeneous population with a large number of signaling pathways even within a single tumor, which limits the possibilities of targeted therapy; the microenvironment of GB enhances their resistance to radiation and chemotherapy; the low immunogenicity of GB cells prevents an adequate immunological response; invasion of the perifocal zone of the brain by glioma cells (including stem cells) significantly complicates effective treatment by surgical resection alone; the blood-brain barrier prevents the achievement of a sufficient concentration in the brain of systemically administered chemotherapy drugs without serious side effects [9]. There is an active search for alternative ways of diagnosis and treatment of GB in the following areas: new technologies of cellular and molecular diagnostics, nanotechnology, the use of stem cells as carriers of ligands and vectors, modern approaches to immunotherapy, technologies of targeted and personalized therapy, etc.

Among the most recent approaches are innovative intraoperative techniques and various methods of treating the "tumor" surgical site. In particular, the following options for the introduction of pharmacological agents are offered: stereotaxic injections of various compounds directly into the tumor using neuronavigation, as well as in combination with intraoperative computed tomography or magnetic resonance imaging (MRI) (chemotherapeutic agents, radioactive iodine, iron oxide nanoparticles); convection-enhanced delivery of compounds using pressure gradient to improve their distribution (chemotherapeutic agents, viral vectors, cytotoxins, radioactive nanoliposomes); implantation of Ommaya or Rickham reservoirs for periodic therapeutic injections over a long period (for chemotherapy, administration of neural stem cells, cell therapy of CAR (chimaeric antigen receptor) T-cells; intra-arterial delivery of high doses of therapeutic agents (antibodies, chemotherapeutic agents, viral vectors) using catheters inserted directly in the arteries involved in the blood supply of the tumor focus [9].

The treatment of MG by alternating electric fields, methods of laser-induced interstitial thermotherapy, magnetic hyperthermic therapy, focused ultrasound, radiofrequency microwave and photodynamic therapy (PDT) are suggested [9, 10].

Photodynamic methods, such as photodynamic diagnosis, fluorescence-guided tumor resection (fluorescence-guided surgery (FGS)), and PDT, are being intensively developed in clinical trials as adjuvant methods of therapy for MG [11, 12].

Invasive glial tumor spread and the degree of surgical resection are difficult to definitively assess during surgery, and areas of tumor cell invasion may be overlooked when using traditional surgical approaches and imaging techniques. In addition, the functional load of certain anatomical areas limits the neurosurgeon in the radicality of the removal, therefore, MG often progressively occur near the marginal area of the surgical cavity as a result of residual glioma cells. A method that enables visual identification of tumor tissue while selectively destroying it will contribute to better surgical removal of MG. Such method is PDT - a two-stage

treatment involving the administration (intravenously, intraperitoneally, locally or orally) of a light-sensitive agent (photosensitizer (PS)) followed by its activation by light rays of a certain wavelength for target lesion of tumor cells.

### **History of photodynamic therapy**

The beginning of using the energy of photochemical reactions is associated with Ancient Egypt, when in the 6th millennium BC vitiligo skin disease was treated with a powder of leaves of parsnip, parsley and St.-John's-wort, which was spread on the affected skin areas, and when exposed to sunlight, light-sensitive components contained in these plants caused a photochemical reaction in tissues, resulting in the appearance of skin pigmentation [13]. Photodynamic procedures are described in ancient books - the Ebers papyrus and the sacred Indian book "Atharvaveda", similar methods (oxygen-free photochemical reactions) were also practiced in ancient China, Thailand, Arab countries (in particular, the use of Aatrillal seed powder (later name - Ammi majus, Chinese cumin)) [14].

Over the past 100 years, the role of photosensitizing agents in the implementation of the antitumor response to photoirradiation has been investigated. In 1900, O. Raab in the laboratory of H. von Tappeiner at the University of Munich established an oxygen-dependent photodynamic effect of a number of natural dyes (in particular, acridine orange) during the solar irradiation of simple unicellular organisms *Paramecium* [14, 15]. H. Jesionek et al. observed an improvement in the skin condition of patients with tuberculosis and syphilis after using an aqueous eosin solution and solar rays or an arc lamp irradiation. H. von Tappeiner and H. Jesionek (1903) first performed PDT in a skin cancer patient using eosin solution as a PS. Affected areas were irradiated with sunlight or an arc lamp. A total of 6 patients were treated, 4 of them achieved complete resorption of lesions with no recurrence within 1 year (1905). It was H. von Tappeiner (1904) who proposed the term "photodynamic reaction" to describe a specific photochemical reaction when irradiation with light and the use of oxygen and a radiation-absorbing dye, resulting in the death of biological systems [15]. Since the discovery of W.H. Hausmann new PS - hematoporphyrin (1908), the sensational experiment of F. Meyer-Betz (1912) with a test of its effect on himself, as well as after the works of A. Policard (1924), who suggested the use of PDT in oncology, focusing on the diagnostic value of fluorescence of tumor-transformed tissues (using hematoporphyrin) [14, 15], the issue of search and synthesis of new PS is relevant. Currently, more than 100 drugs with photosensitizing activity have been synthesized.

A new stage in the development of PDT began with the medical use of lasers (1960s). Since then, PDT is considered an intervention consisting in photochemical destruction under combined exposure to PS and low-intensity laser radiation with an optimal wavelength corresponding to the absorption spectrum of the sensitizer, which significantly increases the intensity of photochemical reactions.

PDT has been developing as a direction of tumor treatment for several decades. In one of the first reports on PDT of glioma tissue (1980), it was assumed that

further improvement of the technique would promote better penetration into tumor tissue and more radical destruction of glioma cells [16]. The use of PDT in neuro-oncology significantly increased the duration of overall and relapse-free survival in patients, reduced the risk of severe neurological complications [17]. In 1996, the results of open phase I/II clinical trials involving more than 310 patients who received PDT after resection of a malignant tumor (primary or recurrent) were published. A clear tendency to increase the average survival rate of patients after surgical removal and single PDT was revealed [18, 19].

In Ukraine, PDT as a new technology for the treatment of oncological diseases began to be developed in the 1970s [20]. Prof. M.F. Gamaliia and Prof. R.E. Kavetsky substantiated the therapeutic effect of laser irradiation by the presence of endogenous porphyrins and molecular oxygen in the peripheral blood as the main photoacceptors, formulating the concept of "photodynamic blood modification" - optimized, mathematically verified low-intensity effect on the blood based on light parameters and doses, the consequences of which are morphological changes of blood elements, local and systemic effects. The developers were awarded the Ukrainian SSR State Prize in the field of science and technology [21].

The problem of using PDT in neuro-oncology began to be addressed in the 1990s at the Institute of Neurosurgery named after acad. A. P. Romodanov, National Academy of Medical Sciences of Ukraine, under the leadership of Prof. V.D. Rozumenko (neuro-oncology department) with the involvement of the tissue culture laboratory (head - Prof. V.M. Semenova). A number of experimental studies [22–28] formed the basis of the clinical development of PDT regimens in neuro-oncology patients [29]. Currently, PDT is a clinically acceptable method of treating various types of cancer, which is constantly being improved [30, 31].

### **Principle of photodynamic therapy and mechanism of action**

Photodynamic therapy was introduced as an option for topical (local) surgical therapy based on cytotoxic effects caused by a chemical photosensitizing agent that selectively accumulates in malignant tumor tissue and is activated by light rays of the appropriate wavelength [32, 33], generating singlet oxygen and free radicals, which trigger photochemical reactions in tumor cells with subsequent destruction of their protein structures. Photodynamic therapy implies photoactivation of PS molecule, which is selectively incorporated into neoplastic cells [34].

Exposure to light rays of a certain wavelength (photoirradiation) activates PS, exciting molecular oxygen to the singlet or triplet state [12]. In the singlet state, energy is converted into heat (internal conversion) or emitted as light (fluorescence). Singlet oxygen has a record long lifetime in the discharged state — 4600 s, or 1.27 h [36]. In the triplet state, reactive oxygen species (ROS) are generated, which react rapidly with macromolecules containing unsaturated double bonds (proteins, unsaturated fatty acids, cholesterol), damaging the membranes of intracellular organelles (mitochondria, lysosomes, endoplasmic reticulum), reacting with DNA, proteins, lipids and

other macromolecules, disrupt multiple cell signaling pathways, extensively destroy DNA, which eventually leads to tumor cells death (through necrosis, apoptosis, autophagy), destruction of the tumor microvascular system, local ischemia, as well as to activation of antitumor immune responses [10, 12, 34, 37–39]. One of PDT targets is macrophages, which produce inflammatory mediators and cytokines (lymphokines, thromboxanes, prostaglandins, tumor necrosis factor, etc.) after photoirradiation, thus significantly affecting the tumor stroma degradation [40]. Moreover, PDT damages endothelial cells, which leads to local thrombosis, vasoconstriction and ultimately to the destruction of the microcirculatory channel. The combination of these effects induces a strong immune response against glioma in the experiment [41]. Therefore, PDT engages the mechanisms of not only direct cytotoxic, but also mediated immunomodulatory antitumor effects, activating the links of innate and acquired immunity.

### **Types of photosensitizers**

Both PSs and photosensitizing precursors are used for PDT [10]. Ideal PSs should be purified from impurities in order to selectively accumulate in tumor tissue and be able to cross the blood-brain barrier. Photosensitizers should also be localized in the tumor tissue without simultaneous accumulation in significant concentrations in healthy tissue and have maximum cytotoxic activity against tumor cells due to absorption of light photons in the 650–700 nm spectral range. It is important that PSs do not cause systemic toxicity and are quickly eliminated from the body [42, 43]. The selectivity of PSs accumulation in the brain is one of the key issues in the problem of PDT effectiveness increase. After administration, PSs accumulate in all body organs, but tumor tissue has a greater affinity for them [35]. The blood-brain barrier, which prevents the penetration of most drugs, does not interfere with PSs. The selectivity of PSs accumulation in brain tissues varies from 3:1 to 50:1 compared to normal tissues [42].

Photosensitizers are divided into 1st, 2nd and 3rd generation molecules.

The 1st generation PSs molecules are porphyrins, namely porfimer sodium and hematoporphyrin derivatives (HpD, a patented combination of monomers, dimers and oligomers derived from hematoporphyrin) [10]. In 1984, T.J. Dougherty et al. [44] conducted a study on the isolation of the active fraction of HpD. The purified mixture of HpD monomers, dimers and oligomers was commercially named Photofrin (USA). In Germany, the analogue is Photosun. These compounds are characterized by strong absorption in the ~400 nm range, but have limited excitation absorption at longer light wavelengths [45]. HpD is an inefficient producer of singlet oxygen, requiring prolonged photostimulation to achieve an adequate therapeutic effect [46]. For PDT with drugs based on HpD derivatives, laser radiation with a wavelength of 628–632 nm is used, while the depth of photoinduced necrosis does not exceed 1 cm. Doses of light energy vary significantly (from 50 to 500 J/cm<sup>2</sup>) and depend on the size and localization of the tumor lesion.

The second generation photosensitizers such as chlorins (thalaporfin sodium and temoporfin (meta-tetra(hydroxyphenyl)chlorin (mTHPC)), pheophorbides,

bacteriopheophorbides, metalloporphyrins, purpurins, phthalocyanines, and 5-aminolevulinic acid (5-ALA) have been developed to overcome the limitations inherent to 1st generation sensitizers. They are usually activated at wavelengths >600 nm and are more effective in singlet oxygen production [10]. Thalaporfin sodium is activated by light with a wavelength of 664 nm [46, 47]. Temoporfin (mTHPC) is the most potent of the clinically available PSs (activated by 652 nm photostimulation), is well tolerated, but has photosensitivity that persists up to 6 weeks after administration. Another mTHPC drug is Foscan (Germany). PDT requires minimal doses (0.1–0.15 mg/kg of body weight) and light energy (10–20 J/cm<sup>2</sup>) at a wavelength of 652 nm. However, its use is associated with side effects (development of tracheal and bronchial stenosis, esophagotracheal fistulas, esophageal perforations). The drug is rapidly excreted from the body, skin toxicity is observed within 1 week [48, 49]. Purlitin (selenium etiopurpurin) (USA) [50] and Lutex (USA) belong to this group of PSs, the feature of which is high selectivity of accumulation in tumors [51]. Photosensitizers based on zinc and aluminum porphyrines and phthalocyanines are being studied for their cytotoxic activity as new antitumor drugs [52].

5-ALA [53], which is physiologically an intermediate product of heme synthesis, is used and commercially available PSs of the 2nd generation. Clinical use is due to oral bioavailability and a favorable safety profile. 5-ALA is highly selective, mainly accumulating in the MG tissue [10]. It is approved by the FDA for PDT of keratosis and fluorescence-guided visualization of tumor tissue during surgical removal of MG [10]. Photodynamic therapy based on 5-ALA is an effective method of adjuvant treatment of MG. 5-ALA induces protoporphyrin IX (PpIX) formation, what is rational to use during FGS of MG due to the selective capture of photosensitizing PpIX by the tumor and minimal skin sensitization [54]. However, insufficient accumulation of PpIX may limit the possibility of using FGS and PDT in the marginal areas of gliomas [55].

Photosensitizers of the 3rd generation are characterized by increased selectivity to tumor cells, which is achieved due to conjugation with modifiers (nanoparticles and antibodies) [10, 46]. When developing the 3rd generation PSs, the focus was on creating prodrugs that are activated only inside tumor cells. The goal of the rational design of the 3rd generation PSs is to reduce side effects while optimizing pharmacokinetics and excitation absorption properties to maximize the effective PDT window. New PSs from the groups of polymethine and tricarbo-cyanine dyes, which have some advantages over compounds of the porphyrin series, are being studied. They are characterized by a light absorption band with a high molar coefficient in the spectrum range of 700–900 nm. Currently, 3rd generation PSs have not yet been approved for clinical use for PDT in patients.

### **Experimental studies of photodynamic therapy**

*In vitro* and *in vivo* experimental studies confirmed the efficacy of PDT of brain tumors using 1st and 2nd generation PSs (HpD, mTHPC, sinoporphyrin sodium, ZnPc and Zn (II) phthalocyanines, tetraminephthalocyanine (TAZnPC), 5-ALA, chlorin E6) either alone or in

combination with fractionated radiation therapy [56], sonodynamic therapy [57], temozolomide (TMZ) chemotherapy (synergistic effect) [58] or EGFR inhibitors [59, 60]. In particular, the antitumor effect of PDT with 5-ALA in SU-DIPG-XIII MG cell cultures in combination with fractionated radiation therapy was shown *in vitro* [56]. Phthalocyanines ZnPc and TAZnPc *in vitro* caused the death of GB cells of T98G, MO59, LN229 and U87-MG lines by apoptosis, which was accompanied by caspase-3 activation, loss of morphology and functions of mitochondria, integrity of lysosomes, externalization of phosphatidylserine and DNA fragmentation of tumor cells [61]. Antitumor effects of combining PDT with sinoporphyrin sodium and sonodynamic therapy *in vitro* in U118 glioma cells were revealed [57]. Promising results were obtained for PDT with chlorin E6 of rat gliomas C6 and 101.8 *in vivo* [62].

Experimental study of PDT *in vitro* using 2nd generation PS - photosens (sulfonated aluminum phthalocyanine) was carried out at the Institute of Neurosurgery in the tissue culture laboratory (Prof. V.D. Rozumenko and Prof. V.M. Semenova). The method of tissue culture was used to obtain the optimal effect of photodestruction of tumor cells in cultures of the transplanted rat brain MG 101.8 and primary human MG cultures. Simultaneously, in a comparative aspect, the reaction of cultured neural cells of the brain of experimental animals to the effect of PS and laser irradiation according to different schemes was studied. It was shown that laser irradiation with a wavelength of 675 nm of cultures of experimental glioma 101.8 pre-incubated in nutrient medium with the addition of photosens (5 µg/ml) causes photodestruction of most tumor cells, which rises with increasing exposure from 120 to 240–300 s (subtotal tumor cell damage and pronounced cell loss). Assessment of 24-h effect of PS on cultures of the normal brain of newborn rats demonstrated the resistance of neurocytes to PS accumulation and only weak incorporation of it into the cytoplasm of some glial cells, which confirms the absence of neurotoxicity when using it [26, 27, 63]. The results of these studies formed the basis of the clinical development of PDT regimens in neuro-oncological patients [29, 64].

In the study of the effect of PDT on gliosarcoma cells of the 9L/lacZ line *in vitro* using photodithazine (PDZ) and Biopdi/IRRAD-LED 660 LED light source at a wavelength of 660 nm showed 100% tumor cells death at different concentrations of PS, which accumulated mainly in the area of nucleus and cytoplasm [65].

Photosens and PDZ at a light dose of 20 J/cm<sup>2</sup> ( $\lambda_{\text{ex}}$  615–635 nm) effectively induced cell death of glioma GL261 and fibrosarcoma MCA205 cells [66]. Photosens was predominantly localized in lysosomes, and photoirradiation-induced cell death was inhibited by apoptosis inhibitor (zVAD-fmk), ferrostatin-1, and ferroptosis inhibitors (DFO), but not by the necroptosis inhibitor necrostatin-1. Instead, PDZ accumulated in the endoplasmic reticulum and Golgi apparatus, and photochemical cell death was inhibited only by z-VAD-fmk. Tumor cells dying due to photosens or PDZ-induced photochemical reaction released calreticulin, HMGB1 and ATP and were effectively absorbed by BMDCs (bone-marrow derived dendritic

cells), which matured, activated and produced interleukin-6, which may be potentially used in cancer immunotherapy [66].

PSs accumulation and PDT efficacy were evaluated *in vitro* in various glioma lines, primary neurons and astrocytes of rats and *in vivo* in the healthy brain and RG2 glioma of Fischer rats [67]. *In vitro* studies on rats showed a significant improvement in the survival of primary rat neuronal cells after PDT, *in vivo* studies showed a decrease in the volume of edema/inflammation on day 10 after PpIX-mediated PDT, confirming the neuroprotective effect. Moderate hypothermia increased PpIX fluorescence in the tumor by 5 times and the average survival time of rats after PDT without affecting normal brain structures [67].

In an *in vivo* study, fox1 rnu/rnu rats with U87 GB after intraperitoneal injection of 5-ALA (100 mg/kg) were interstitially irradiated to the tumor in two regimens: with high (30 mW) or low (4.8 mW) radiation intensity. Higher intensity and fractionation of irradiation caused a greater degree of tumor necrosis [54].

#### **Experimental approaches to enhancing the effectiveness of photodynamic therapy**

Various preconditioning regimens are used to enhance the fluorescence of PSs. Thus, selective enhancement of PpIX fluorescence and PDT with 5-ALA was achieved by pre-conditioning glioma cells and control astrocytes with calcitriol for 48 h before incubation with 5-ALA. The authors suggest that the combined treatment of glioma tumor cells with calcitriol using 5-ALA may be an effective and selective therapeutic method to improve the quality of PpIX fluorescence induced by 5-ALA [55].

A combination of different treatment methods is being investigated to increase the effectiveness of PDT. The synergistic effect of PDT and chemotherapy has been proved. It has been shown that the drug gefitinib, EGFR inhibitor developed for treatment of breast and lung cancer, can enhance the photodynamic effect in brain tumor cells through gefitinib-mediated inhibition of the ATP-binding cassette transporter ABCG2, which prevents the efflux of PS from brain tumor cells and enhances effect of PDT [59]. The combined effect of another EGFR inhibitor, lapatinib, has been also studied. Its administration 24 h before PDT and for 3–5 days after PDT significantly increased PpIX accumulation and the decrease in LD<sub>50</sub> after PpIX-mediated PDT in U87 and U87vIII MG cell lines *in vitro* and increased the survival of U87 and GSC-30 glioma-grafted rats compared with therapy with lapatinib or PDT alone [60]. The combination of TMZ with PDT significantly increased TMZ concentration in glioma tissues, enhanced tumor cell apoptosis, and increased the average survival rate of rats with transplanted glioma [58].

One of the markers of tumor vessels in glioma is an extra fibronectin domain A (ED A). The use of antibodies against ED A fibronectin promoted the accumulation of PS (F8-SIP) in the microvascular bed of the grafted glioma SF126 in nude mice [68]. Subsequent PDT induced microvascular stasis and thrombosis with decreased functional density and decreased glioma growth with restoration of microcirculation 4 days after treatment. Repeated use of PDT prevented microvascular recovery and resulted in a prolonged anti-glioma effect [68].

The nature and role of ROS-mediated damage to tumor cells as a result of PDT have not been fully elucidated. In this regard, the response of nitric oxide (NO) signaling pathways in tumor cells is worth attention, since the growth and proliferation of MG stem cells depend on the activation of inducible NO synthase (iNOS) [69], and iNOS inhibitors can play a role in increasing PDT efficiency [70]. Thus, iNOS/NO of U87 glioblastoma cells counteracts the cytotoxic effects of non-ionizing PDT and stimulates the growth and migration activity of surviving tumor cells [71], and iNOS-derived NO in GB cells causes resistance to 5-ALA-based PDT [72].

It is also known that tight intercellular junctions (GJIC), formed by connexin (Cx) 43, can improve "death signal" transmission between cells, increasing the cytotoxic effect of chemotherapeutic agents, gene therapy, and PDT [73]. Photodynamic therapy was more effective in Cx43-transfected HeLa cells and Cx43-expressing U87 glioma cells at high cell density. This effect was significantly suppressed in the absence of Cx43 expression or Cx43-GJIC blockage. In addition, in the presence of Cx43-GJIC, photofrin-PDT reduced the mass of tumor xenografts *in vivo*. Increased PDT efficiency associated with Cx43-GJIC was correlated with ROS-mediated stress signaling pathways, Ca<sup>2+</sup> and lipid peroxidation [73]. Therefore, strategies to enhance Cx43 expression or Cx43-GJIC function may increase tumor cell sensitivity to PDT and treatment efficacy.

#### **Clinical studies of photodynamic therapy**

As mentioned above, PDT as a direction of tumor treatment has been evolving for several decades. One of the first reports on PDT of glioma tissue in 1980 predicted that further improvement of the technique would facilitate better penetration into tumor tissue and more radical destruction of glioma cells [16]. Although a large number of PDT trials have been conducted for the treatment of malignant brain tumors, most of them are uncontrolled phase I or II studies, which differ greatly in their design (choice and dose of PS, choice and dose of photoirradiation, method of irradiation and use of additional techniques, choice and the formation of groups of patients according to diagnoses, indicators and interpretation of findings, etc.), which makes the overall analysis and assessment of treatment effectiveness extremely difficult.

In 1996, the results of open phase I and II clinical trials, which involved more than 310 patients with primary or recurrent malignant tumors, were published. Patients received PDT after tumor resection. A clear tendency was found to increase the average survival rate of patients after surgical removal and single PDT [18]. Based on the clinical experience of PDT for the treatment of gliomas in more than 350 patients using HpD (since 1987) and borated porphyrin (since 2001), a group of researchers from the Royal Melbourne Hospital reported an overall survival of patients with rediagnosed and recurrent GB 28.0 and 40.0% after 2 years and 22.0 and 34.0% after 5 years. A meta-analysis of data from more than 1,000 patients with MG showed that median survival after PDT for rediagnosed and recurrent MG was 16.1 and 10.3 months, respectively [74]. According to the results of a phase II clinical trial (JMA-IIA00026, Japan) of intraoperative PDT with talaporfin sodium

[11] involving 27 patients with newly diagnosed or recurrent primary parenchymal brain tumors, among the 22 patients who were subsequently involved in the study cohort, the 12-month total survival and 6-month progression-free survival after surgery and PDT were 95.5 and 91.0%, respectively, in 13 patients with newly diagnosed GB - 100%. Side effects on the skin that can be associated with the use of talaporfin sodium were reported in 7.4% of patients. Skin photosensitivity test results were relatively mild and completely disappeared within 15 days after administration of photosensitizer in all patients [11].

Two photosensitizing agents have been studied primarily in MG: porfimer sodium (Photofrin, FDA-approved for the treatment of esophageal cancer) [75] and 5-ALA (FDA-approved for imaging of GB cells during surgery) [13]. In clinical trials, PDT was usually combined with other therapeutic interventions or manipulations.

Simultaneous use of fluorescence-guided surgery (FGS) and PDT allows both imaging of tumor cells and selectively destruction of them [34]. For intraoperative and postoperative PDT, special devices based on fiber-optic guided technique have been developed. Regardless of PDT, PSs are used for auxiliary detection of the tumor boundaries in order to maximize its removal during FGS (5-ALA, fluorescein, indocyanine green, endogenous fluorophores) [76].

One of the main caveats of PDT is that the laser beam must reach the cells containing the sensitizing agent. Commonly used lasers have a wavelength range of 630–690 nm, and the penetration depth rarely exceeds 5.0 mm in most tissues [77]. However, the effects of PDT may be greater, as MRI data show the mean penetration depth of 9.1 mm [78] and postmortem histopathological analysis revealed penetration up to 12.7 mm [79]. However, deeply located malignant cells remain completely unaffected. In addition, according to [78], 75% of cases of continued tumor growth/recurrence were observed in the PDT area, which casts doubt on the long-term effect even in the resection cavity. However, given the clinical availability of PSs and the low risk level of this therapy, further studies of their use in GB are needed [80].

A small number of clinical trials of PDT (porfimer sodium, 5-ALA) in the treatment of MG have been conducted, of which several have been discontinued or withdrawn (n=3) or their status is unknown (n=3), 4 are ongoing [81].

In a phase III trial using porfimer sodium for the treatment of GB (n=27) [82], patients who received five daily PDT sessions with an implanted laser in the resection cavity had better survival compared to those who did not receive PDT, but patients who received PDT underwent resection with the help of FGS, which in itself improves treatment outcomes [13]. In addition, 15% of patients in both groups received TMZ, which limited the generalizability of the study results.

Another trial (phase I) used 5-ALA for FGS and intraoperative PDT in 20 patients with recurrent GB [78]. After 5-ALA FGS, 1–4 cylindrical laser diffusers were inserted into the resection cavity and PDT was performed under general anesthesia for 60 min (635 nm, diffuser 200 mW/cm) with continuous irrigation

to maintain optical clarity and ventilation with 100% oxygen. According to MRI performed after 24 hours, 14 days and every 3 months thereafter, infection at the surgical site was noted in 1 patient was after 6 months as the only side effect. In 16 (80%) of 20 cases, cytotoxic edema along the resection margin was detected, which regressed or disappeared after 4–5 months. The median progression-free survival was 6 months (95% confidence interval (CI) – 4.8–7.2 months), which is comparable to the standard treatment for recurrent GB [83] and confirms the innovativeness and safety of FGS and PDT combination with the use of 5-ALA as a method of local tumor control promising for tumor progression-free survival [78].

Preliminary results of a clinical study (phase I) Intraoperative Photodynamic Therapy of GBM (INDYGO) clinical trial (NCT03048240) involving 10 patients with primary GB, started in 2017 at the University Hospital of Lille (France), proved the safety and feasibility of using PDT based on 5-ALA in primary GB [80]. The standardized treatment approach involved maximal resection (close to total or gross total tumor resection) under 5-ALA FGS control followed by intraoperative PDT. In the postoperative period, patients received adjuvant therapy (according to the Stupp protocol). Further follow-up included clinical examinations and brain MRI every 3 months until tumor progression and/or death. No unacceptable or unexpected toxic or serious side effects were reported. At the time of the interim analysis, the 12-month recurrence-free survival rate was 60% (median 17.1 months), the 12-month overall survival rate was 80% (median 23.1 months) [80]. Therefore, intraoperative administered PDT with 5-ALA immediately after maximal resection as adjunctive therapy in primary GB is safe and may help reduce the risk of recurrence by targeting residual tumor cells in the resection cavity.

Other ongoing studies are using stereotactic PDT in primary (NCT03897491) and recurrent (NCT04469699) GB, as well as intraoperative and interstitial PDT (iPDT) in primary GB (NCT04391062, NCT03897491).

In the study [84], MRI results were analyzed in 11 patients with primary GB after iPDT. The procedure implied the use of 5-ALA for selective metabolism of protoporphyrin IX (PpIX) in tumor cells and irradiation using interstitially located optical cylindrical diffuser fibers (2–10 fibers, diffuser length 2–3 cm, 200 mW/cm, 635 nm, 60 min irradiation), as well as intraoperative spectral online monitoring. MRI results indicated PDT-induced hemoglobin deoxygenation, methemoglobin formation and were consistent with *in vitro* experiments.

The results of a retrospective study of the Medical Clinic of the University of Munich (Germany) on the analysis of the risk profile of iPDT using induced 5-ALA PpIX as a cytotoxic PS in a large monocentric cohort of patients with local tumor recurrence after standard therapy (who were consecutively treated between 2006 and 2018) proved its promise in local recurrences of MG [85]. 47 patients were treated, 44 of them were evaluated retrospectively. Recurrent gliomas included 37 GB (WHO grade 4) and 7 anaplastic astrocytomas (WHO grade 3). Methylated O-6-methylguanine-DNA methyltransferase (MGMT) was detected in 30 (68.2%) tumors, and wild-type isocitrate dehydrogenase (IDH) in 29 (65.9%). 26 (59.1%) patients received treatment

after the first relapse, 9 (20.5%) after the second, and another 9 (20.5%) after the third or subsequent relapse. The mean iPDT target volume was 3.34 cm<sup>3</sup> (0.50–22.8 cm<sup>3</sup>). Severe neurological deterioration lasted more than 6 weeks in only one patient. Median time-to-treatment-failure (TTF) was 7.1 months (95% CI 4.4–9.8 months), median post-relapse survival 13.0 months (95% CI – 9.2–16.8 months). The 2-year and 5-year post-relapse survival rates were 25.0 and 4.5%, respectively. Response to treatment was not significantly associated with patient characteristics, treatment-related factors, or molecular markers. The promising result and acceptable risk profile deserve further prospective evaluation, particularly to determine the mechanisms and prognostic factors of a favorable response to the treatment of MG [85].

In the study [86] the effectiveness of PDT with talaporfin sodium in patients operated on for recurrent GB was retrospectively evaluated. The average relapse-free survival in patients with PDT (n=72) and the control group (n=38) after the second surgery was 5.7 and 2.2 months, respectively (p=0.0043), the average overall survival after the second surgery was 16, 0 and 12.8 months, respectively (p=0.031). In the PDT group, there was no significant difference in relapse-free and overall survival between patients previously diagnosed with GB and those with malignant transformation to GB from less malignant glioma variants. Therefore, the use of PDT in the progression of GB may have potential survival advantages, and its effectiveness is independent on pre-recurrence pathology [86].

In another study [87] the clinical and surgical data of the treatment of patients with primary GB, divided into three groups were compared: operated on with the use of 5-ALA FGS followed by BCNU plates implantation (n=20, group I), those operated on with implantation of BCNU plates (n=42, group II), operated on with the use of 5-ALA FGS (n=59 patients, group III). In group I patients, life expectancy exceeded 3 years in 15% of cases, the median relapse-free and overall survival was 11 and 22 months, respectively. Patients implanted with BCNU plates had a significantly higher survival rate after tumor removal with 5-ALA FGS (22 months with 5-ALA (Group I) vs. 18 months without 5-ALA (Group II), p<0.0001). Patients in group I had an increased survival compared to group III (22 months with BCNU plates vs. 21 months without BCNU plates, p=0.0025). Plate-related adverse events were not significantly increased with 5-ALA FGS (20% with 5-ALA and 19% without 5-ALA) and did not affect survival outcome. This study confirms the synergistic effect in patients with primary GB of 5-ALA FGS technology and BCNU plates implantation without increasing the incidence of adverse effects [87].

Therefore, PDT as a treatment method has a clearly focused effect aimed at selectively increasing the tumor destruction area during surgery, which improves the survival time and quality of life of patients with MG. The main advantage of the PDT method is its high efficiency, organ-sparing technique and low systemic toxicity. In published studies of PDT effectiveness, there is no information on the development of resistance to multiple PDT sessions, which suggests the possibility of re-treatment of tumor cells not removed during

surgery. The accumulated experience of PDT application in oncological diseases suggests that PDT is one of the most effective methods of stopping the local spread of tumor cells, but it is necessary to optimize radiation doses, the number of treatment procedures and the intervals between them. The effectiveness of photodynamic damage to a sensitized cell is determined by the intracellular concentration of the sensitizer, its localization in the cell, photochemical activity, and the dose of laser irradiation. It is necessary to carry out fundamental research with the development of new experimental models of PDT, in which multiple effects on the tumor will be applied.

### Information disclosure

#### Conflict of interest

The authors declare no conflict of interest.

#### Ethical approval

This article is a literature review, therefore no ethics committee approval was required.

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