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Intraoperative Sono-photodynamic Therapy with Photolon in Animal Experiments and Promising Results of Phase I Clinical Study in Patients with Recurrent Malignant Gliomas

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Abstract

Aim: Evaluate the efficacy and safety of intraoperative sono-photodynamic therapy (iSPDT) with photosensitizer photolon in rats with orthotopic C6 glioma and then to test this therapy in patients with recurrent malignant gliomas.

Materials and methods: The experimental study was performed on 5 groups of rats bearing C6 glioma: untreated control, tumor resection (TR) only, TR+intraoperative sonodynamic therapy (iSDT), TR+intraoperative photodynamic therapy (iPDT) and TR+iSPDT. Photolon was injected intravenously shortly before TR that was followed by iSDT and iPDT. The criterium of treatment effeicacy was median overall survival (OS) of the animals. The clinical Phase I study comprised 15 patients with recurrent malignant gliomas. The first stage of the treatment was total/subtotal TR followed by intravenous administration of photolon; then the tumor bed was consequtively exposed to ultrasound (1.04 MHz; 1 W/cm^2; 10 min.) and laser irradiation (50-100 J/cm^2) 0.5 h after the start of photolon infusion. Within 4 weeks after discharge from hospital all patients underwent chemotherapy. The toxicity of anticancer therapies was evaluated on the basis of frequency and severity of adverse reactions accounted in accordance with CTCAE (Version 4.0). The criteria for assessing antitumor efficacy were: MRI images at 3 and 6 months after iSPDT treatment, median OS and post-iSPDT median times.

Results: TR+iSPDT of orthotopic C6 glioma increased the median OS of rats to 38 days in comparison with 18 days in the TR group (p=0.001); the combined effect of iSDT and iPDT was approximately additive. In the human patients, treatment-related toxicities were of grade I/II only. The median OS of died patients from first diagnosis was 23.9 months in iSPDT and 12.1 months in control group, respective (p=0.004). The post-iSPDT median survival was 8.2 month, while in the control group (without iSPDT) it was 5.8 month (p=0.012).

Conclusions: iSPDT with photolon may be considered as a fairly safe and potentially effective option for the adjuvant management of malignant brain tumors.

Keywords: Intraoperative sono-photodynamic therapy; Photolon; Malignant glioma recurrence

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Introduction

Malignant brain tumors are a serious health and social problem. The frequency of occurrence of this disease is 85-90% of all the neoplasms of the central nervous system. The most common tumors with the least favorable prognosis include anaplastic astrocytoma (WHO grade III) and glioblastoma multiforme (WHO grade IV). The one most common form of malignant glioma is glioblastoma [1]. The main treatment for highly malignant gliomas is surgery [2,3] in combination with external beam

radiation therapy [4,5] and chemotherapy with temozolomide [6,7]. In spite of obvious achievements of the medical science of the last decades, the results of treatment of patients with glioblastoma grade IV remain disappointing. According to numerous epidemiological studies, 5-year survival in patients with this pathology is an average of 3-5%, and the median overall survival from the time of histological verification varies from 12.6 to 19 month [8-10]. Today, in many countries there is an intensive search and development of new methods of treatment of malignant gliomas allowing to increase the overall survival of patients with this disease.

One of these is sono-photodynamic therapy (SPDT), which is a treatment method based on the significant increase of the cytotoxicity of drugs combined with ultrasound (US) and photoirradiation of the tumor tissue. According to numerous studies of sono-photochemical reactions include a direct interaction of excited molecules with the help of ultrasonic radiation, the photosensitizer (PS) on the substrate and forming transient radicals that react with oxygen.

Interaction initiates a complex cascade of free radicals, such as singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}OH$), hydrogen peroxide (H_2O_2) and superoxide anion radical ($^{\cdot}O_2^-$), causing the development of oxidative stress syndrome. As a result, SPDT effectively induced glioma-cell apoptosis and necrosis. The two possible mechanisms might be: a) promoting mitochondria to release Cyto-C and activate Caspase-3, then to initiate apoptosis; b) the destroying of microvessels, inhibition of angiogenesis and the induction of ischemia and anoxia of glioma cells, resulting in ischemic necrosis [11-17].

Our *in vitro* and *in vivo* studies showed a high antitumor efficacy of SPDT [18-20]. In the *in vitro* study on C6 glioma cells we showed that photolon produced a pronounced sonosensitizing effect and increased the cytotoxic effect of US by 1.5-2.3-fold [18]. In the *in vivo* study on subcutaneously transplanted glioma C6, most pronounced antitumor effect with the maximal (100%) tumor necrosis was achieved in 2.5 h after intravenous administration of photolon with ultrasonic exposure at a frequency of 0.88 MHz and pulse intensity of 0.7 W/cm^2 for 10 minutes, followed by photoirradiation at a dose of 100 J/cm^2 [19,20]. Obtained in the experiment data formed the basis for further study of the efficacy and safety of this method in the clinic.

The aim of this study was to evaluate the results of phase I clinical testing method intraoperative sono-photodynamic therapy with photosensitizer photolon in patients with recurrent malignant gliomas.

Materials and Methods

Experimental study design

Cell culture: Experimental rat C6 glioma was obtained from the tumor strains collection of the Russian Collection of Cell Cultures, Cytology Institute of Russian Academy of Sciences, St. Petersburg and was passed by serial transplantation. C6 glioma cells were grown at 37°C, 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and 50 g/ml canamycin. Cells in exponential growth were harvested by EDTA/Trypsin for 5 min. at 37°C. The trypsinization was stopped by medium with 10% FCS and the cells centrifuged. The pellets were then resuspended in medium without any supplement, at the concentration required by the different study groups.

Animals: Thirty eight white randomly bred rats of both sexes weighing 150 ± 30 g obtained from vivarium of the NN Alexandrov National Cancer Center of Belarus (Minsk) were divided into 5 groups: intact control (n=10); tumor resection (TR, n=7); tumor resection+intraoperative sonodynamic therapy (iSDT, n=7); tumor resection+intraoperative photodynamic therapy (iPDT, n=7); tumor resection+intraoperative sono-photodynamic therapy (iSPDT, n=7). The animals received a standard diet and had permanent access to water. Before treatment, animals were anesthetized by intramuscular introduction of a solution of droperidol (5.0 mg/kg) and fentanil (0.05 mg/kg).

All manipulations were carried out according to the international scientific ethic standards of the quality of planning and carrying out animal investigations, according to Methodic instructions for carrying out preclinical investigations of pharmacokinetics of pharmacologic substances and drugs» presented in the «Good Laboratory Practice TKP 125-2008» (Health Ministry of Republic Belarus, Minsk).

Orthotopic tumor model: Through a midline sagittal incision, a bur hole was made 1.5 mm in diameter with a dental drill at a point 2 mm posterior to the right coronal structure and 4 mm lateral to the sagittal midline. Using sterile technique rats were implanted with C6 glioma cells (600000 cells/15 µl) through a 3 mm craniectomy over the left hemisphere anterior to the coronal suture. Using a syringe, cells were slowly injected into the cortex 3.5 mm deep, 3 mm to the right and 2.5 mm anterior of the bregma. The syringe was slowly withdrawn after 5-7 min, the cranioectomy covered and the incision closed with coated braided synthetic absorbable sutures. Animals were daily examined for alertness, focal motor deficits, gait disturbance and responses to contact. Animals were treated for 9-11 days after tumor cell inoculation.

Photosensitizer (PS): Chlorin e6 conjugated with polyvinyl pyrrolidone (Photolon®, RUE «Belmedpreparaty», Minsk, Republic Belarus) was injected in tail vein in standard dose 2.5 mg/kg. The experimental research on PS pharmacokinetics in healthy and tumor tissues of rat brain was carried out on 10 laboratory animals. The photolon accumulation level was measured with spectral fluorescence technique using «LESA-01-Biospek» spectrum analyser (Moscow; Russian Federation, λ=632.8 nm).

Operation procedure: Animals were anesthetized and fixed in operation device, the skull was exposed and 0.5 cm diameter craniotomy was drilled over the right hemisphere 3 mm to the right and 2.5 mm anterior of the bregma. Five minutes later, the C6 glioma resection was exposed by a microneurosurgical method. After all types of treatment procedures, the hole was sealed with bone wax and the skin was sutured. The animals were then returned to cages and kept in normal room light continually monitored for any signs of neurological deficit.

Intraoperative sonodynamic therapy (iSDT: PS+US): Tumor insonation procedure was performed 0.5 h after tumor resection and 1 h after photolon administration using BTL-5710 Sono (BTL Industries Limited, Great Britain) with an emitter of 5 cm^2, 1 MHz ultrasound frequency in a continuous mode with 0.7 W/cm^2 intensity for 10 min employing stable techniques.

Intraoperative photodynamic therapy (iPDT: PS+photoirradiation): Photoirradiation of tumors was carried out after iSDT using diode laser with 660 ± 5 nm wavelength («IMAF-AXICON», Minsk, Republic Belarus) at doses of 50 J/cm^2 with 0.51 W/cm^2 fluence rate. The output was 0.1 W, the light spot diameter 0.5 cm, irradiation time was 2 min.

The criteria for efficacy evaluation were mean survival time (MST) and median overall survival (OS) of the animals in the study groups vs. the TR group.

Statistical processing of the results: Statistical processing of survival outcomes was done with the Kaplan-Meier method using log-rank test (statistical software package Statistica 8.0). Statistical significance of differences was relevant at $p<0.05$.

Clinical study design

The work is based on the analysis of treatment results of 25 patients with recurrent forms of malignant gliomas who received treatment in Department of Neurooncology and Neurosurgery of NN Alexandrov National Cancer Centre of Belarusian the period May 2014 - January 2016.

The control group: The control group included 10 patients with recurrent glioblastoma Grade IV: 6 women (60%) and 4 men (40%). The mean age was 55.4 ± 9.9 years **(Table 1).**

All patients included in the study had previously undergone surgical intervention in the volume of total/subtotal resection of the primary tumor focus with courses of adjuvant chemotherapy with carmustine (2 mg, intravenously) and/or lomustine (40 mg, orally) 3-4 weeks after completion of surgical intervention (number of chemotherapy courses: 3-7).

The main group: The main group included 15 patients (n=12 -with recurrent glioblastoma Grade IV and n=3 -with recurrent astrocytoma Grade II-III: 7 women (47%) and 8 men (53%). The mean age was 49.5 ± 9.7 years **(Table 2).**

Patients included in the main group were performed total or subtotal removal of tumor recurrence. 0.5 hours before the end of the operational phase the patients are intravenously infusionsolution photolon in the dose of 2 mg/kg and local ultrasonic treatment was performed at a frequency of 1 MHz, intensity of radiation 1 Wcm^2 and power 3 W for 10 minutes. After hemostasis, as second stage, photoirradiation of the bed and walls of the removed tumor was performed at the exposure dose of light 50 (n=5), 75 (n=5) and 100 (n=5) J/cm^2. Depending on the number of exposure fields of laser radiation, absorption dose ranged 240-729 J with the total time of PI 9-39 minutes.

As the next step, 3-4 weeks after the treatment (surgical intervention+iSPDT), the patients received chemotherapy as patients of control group.

Follow-up and evaluation of treatment effects: All patients were performed brain MRI with contrast enhancement to monitor the effectiveness of the treatment and after 3 and 6 months.

Assessment of tolerability and safety of the treatment was carried out for 30 days after the treatment on the basis of data on adverse events and reactions revealed in the course of treatment, their nature, frequency and severity. Given the specific characteristics of the disease and the treatment, the following side effects were assessed (CTCAE, Version 4.0, http://www.meddramsso.com).

Statistical analysis: To estimate the patients' survival rates, Kaplan-Meier method was used. The comparative survival analysis was performed with non-parametric log-rank test. Differences were considered statistically significant at a significance level of $p<0.05$.

The following parameters were studied:

- frequency and severity of complications and adverse reactions after treatment (%);
- median overall survival (OS; month);
- post-iSPDT median survival (month);
- Indicators of 6-, 12-, 18- and 24-month survival rate after diagnosis verification (%).

The calculation of statistical indicators was performed with «STATISTICA 8.0» program.

Experimental Results

At the first stage of the research, to evaluate the time of treatment administration after photolon injection, the dynamics of its accumulation was investigated in healthy and tumor tissues of the brain on days 12-14 after tumor cell implantation. The photolon accumulation level was measured 0, 1, 3, 6 and 24 h after injection. The results are presented in **Figure 1.**

Based on the data on photolon accumulation dynamics, C6 glioma treatment procedures (iSDT, iPDT and iSPDT) were performed with the maximal value of photosensitizer fluorescence signal in tumor tissue. Photolon concentration was demonstrated to reach its maximal value 0.5 h after PS intravenous injection into C6 glioma tissues. Results antitumor effectiveness of the methods of treatment of C6 glioma in rats are presented in **Table 3.**

Table 1 Data on the control group patients included in the study.

Patient	Patient information			
	Sex	Age, Years	Tumor location	Morphological diagnosis
I.	F	45	Frontal lobe	Glioblastoma, Grade IV
Z.	m	65	Temporal lobe	Glioblastoma, Grade IV
O.	f	54	Temporal/parietal lobe	Glioblastoma, Grade IV
B.	f	57	Frontal lobe	Glioblastoma, Grade IV
B.	f	57	Temporal/parietal lobe	Glioblastoma, Grade IV
K.	m	49	Temporal/parietal lobe	Glioblastoma, Grade IV
G.	m	66	Frontal lobe	Glioblastoma, Grade IV
R.	f	72	Temporal/parietal lobe	Glioblastoma, Grade IV
R.	f	43	Temporal lobe	Glioblastoma, Grade IV
A.	m	46	Temporal/parietal lobe	Glioblastoma, Grade IV

Table 2 Data on the main group patients included in the study.

Patient	Patient information			
	Sex	Age, years	Tumor location	Morphological diagnosis
K.	m	51	Temporal/Parietal Lobe	Glioblastoma, Grade IV
R.	m	57	Temporal/Parietal Lobe	Glioblastoma, Grade IV
P.	m	37	Temporal Lobe	Glioblastoma, Grade IV
C.	f	62	Temporal/Parietal Lobe	Glioblastoma, Grade IV
X.	m	57	Parietal/Occipital Lobe	Glioblastoma, Grade IV
K.	f	54	Temporal Lobe	Anaplasticoligoastrocytoma*, Grade III
I.	m	45	Frontal Lobe	Glioblastoma, Grade IV
C.	m	41	Temporal/Parietal Lobe	Fibrillar Astrocytoma*, Grade II
Y.	m	54	Temporal Lobe	Glioblastoma, Grade IV
G.	f	30	Temporal/Parietal Lobe	Fibrillar Astrocytoma*, Grade II
M.	f	50	Temporal Lobe	Glioblastoma, Grade IV
K.	f	35	Temporal Lobe	Glioblastoma, Grade IV
S.	f	60	Temporal/Occipital Lobe	Glioblastoma, Grade IV
G.	m	55	Temporal Lobe	Glioblastoma, Grade IV
S.	f	54	temporal/parietal lobe	Glioblastoma, Grade IV

*with Glioblastoma Grade IV Transformation.

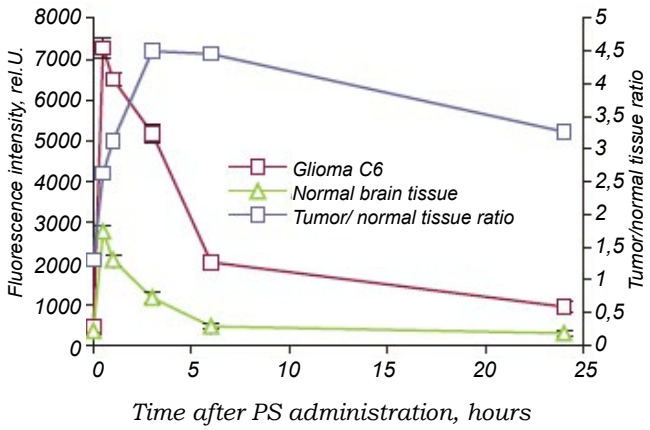

Figure 1 The dynamics of photolon fluorescence intensity in healthy brain tissue and orthotopicglioma c6 after its 2.5 mg.kg intravenous injection.

Table 3 MST and median survival rates in control and main groups.

Groups	Number of animals, n	Efficacy parameters		p
		MST, M ± m, days	Median survival (min-max), days	
Intact control	10	13.4 ± 2.7	14 (10–18)	–
TR	7	17.8 ± 2.6	18 (14–21)	0.01
TR+iSDT	7	25.2 ± 3.7	25 (19–28)	0.0001
TR+iPDT	7	29.8 ± 4.2	30 (24–34)	0.0001
TR+iSPDT	7	39.4 ± 4.5	38 (33–45)	0.0001

The authors demonstrated that median OS rates significantly higher in laboratory animals with orthotopic C6 glioma treated with TR+iSPDT, compared with the following groups: TR (p=0.001); vs. TR+iSDT (p=0.01); vs. TR+iPDT (p=0.03). Our data on MST and median survival of the animals demonstrated that iSPDT with photolon improved results of treatment outcomes in laboratory animals with experimental gliomas.

Clinical Results

Treatment safety in the main group

Evaluation of the frequency and severity of adverse reactions and complications. PS introductionwas not accompanied by violations of vital functions in any case. In all patients, the postoperative period was favorable, no serious complications were found. 3 out

of 5 patients from group 100 J/cm^2 has been noted moderately severe headache (CTCAE, version 4.0; grade II) in the early postoperative period. The nature and frequency of adverse events recorded during the patients' hospital time are presented in **Table 4.** According to multislice computed tomography 24 hours after treatment, no signs of intracranial bleeding in the postoperative cavity were identified. No manifestations of cutaneous phototoxicity (itching, pasty skin) were noted.

Treatment efficacy in the main group

Frequency evaluation of tumor stabilization based on MRI data. In order to evaluate the immediate results after exposure in patients in the study group, intravenous contrast MRI was performed in 3 and 6 months after the treatment **(Table 5).**

The clinical case is an example. Patient, male, 57 years old, AC 1858/2014. Clinical diagnosis: glioblastoma Grade IV left temporal/parietal lobe. Recurrence form after surgical intervention, intraoperative PDT, external beam radiation therapy (2 Gy, 60 Gy) and 3 courses of temozolomide 160 mg/day.

Total surgical resection of the recurrent tumor was performed. 0.5 hour before the end of the surgical phase intravenous drip injection with photolon started at a dose of 2 mg/kg (200 mg). 0.5 hour after infusion, the resected tumor bed was filled with 0.9% saline. Local ultrasonic treatment was carried out at frequency 1 MHz pulses, radiation intensity 1 W/cm^2, 3 W for 10 minutes, by 2 fields with ultrasound therapy apparatus. At second stage, photoirradiation was performed in the light exposure dose of 50 J/cm^2 (total absorption dose 486 J) with laser apparatus

Table 4 Frequency and severity of adverse reactions after iSPDT.

Toxicity*	Exposure dose, J/cm^2								
	50 J/cm^2 (n=5)			75 J/cm^2 (n=5)			100 J/cm^2 (n=5)		
	Grade I	Grade II	Grade III	Grade I	Grade II	Grade III	Grade I	Grade II	Grade III
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Headache	**2** (40%)	0	0	**1** (20%)	0	0	0	**3** (60%)	0
Syncope	0	0	0	0	0	0	0	0	0
Tremor	0	**1** (20%)	0	0	**1** (20%)	0	0	0	0
Edema cerebral	0	0	0	0	0	0	0	**1** (20%)	0
Ataxia	0	0	0	0	0	0	0	0	0
Intracranial hemorrhage	0	0	0	0	0	0	0	0	0
Meningismus	0	0	0	0	0	0	0	0	0
Infections	0	0	0	0	0	0	0	0	0
Nervoussystemdisorders	0	0	0	0	0	0	0	**1** (20%)	0
Skinphototoxicity	0	0	0	0	0	0	0	0	0

*Grade 1 to 3 included; Grades 4 and 5 were not revealed; ****Grade I*** mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated; **Grade II** oderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living; **Grade III** Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care activities of daily living.

Table 5 The results of MRI studies in main group.

Pat.	Patient information						
	Sex	Diagnosis	extent of surgery	ED[1], J/cm^2	MRI scans (3 months)	MRI scans (6 months)	Follow-up, months
K.	M	GB[4]	PR[2]	50	Progression	Progression	9 mon., died
R.	M	GB	PR	50	Stabilization	Stabilization	23 mon., alive
P.	M	GB	PR	50	Progression	Progression	5.5 mon., died
C.	F	GB	PR	50	Progression	Progression	6.5 mon, died
X.	M	GB	PR	50	Stabilization	Stabilization	11 mon., died
K.	F	AOA[5]	CR[3]	75	Stabilization	Stabilization	21 mon., alive
I.	M	GB	PR	75	Stabilization	Regress	9 mon., died
C.	M	FA[6]	CR	75	Stabilization	Stabilization	6.5 mon., alive
Y.	M	GB	PR	75	Stabilization	Regress	12 mon., alive
G.	F	FA	CR	75	Stabilization	Stabilization	6 mon., alive
M.	F	GB	PR	100	Progression	Progression	5 mon.,alive
K.	F	GB	CR	100	Stabilization	Stabilization	5 mon., alive
S.	M	GB	CR	100	Progression	Progression	4 mon., alive
G.	M	GB	CR	100	Progression	Progression	3 mon., alive
S.	F	GB	CR	100	Stabilization	Stabilization	3 mon., alive

[1]ED – Exposure dose; [2]PR – Partial resection; [3]CR – Complete resection; [4]GB – Glioblastoma grade IV; [5]AOA – Anaplastic oligoastrocytoma (grade III) with glioblastoma grade IV transformation; [6]FA – Fibrillarastrocytoma (grade II) with glioblastoma grade IV transformation.

«UPL PDT» (Imaf Axicon, Belarus, λ=660 ± 5 nm). Laser light was delivered on the resected tumor bed in a continuous mode with a fiber with a micro lens. Photoirradiation had the following parameters: output power 0.3 W; power density 0.1 W/cm^2; time of one field 9 minutes, the number of fields 3.

30 days after treatment the patient received 2 courses of chemotherapy with carmustine (2 mg) intravenously over 1-2 hours in an outpatient setting.

According to the follow-up control MRI 6 months after the treatment, no signs of subsequent tumor growth were observed **(Figure 2).**

By January 01, 2016, follow-up observation after the diagnosis verification is 21 months; 6 months' disease-free period after the second surgery, systemic chemotherapy with carmustine and iSPDT (based on MRI data).

Overall survival (for patients with glioblastoma Grade IV). The median OS for main group patients (n=7) amounted to 23.9 month [95% CI=12-35 month], it being 12.1 month in the control group (without iSPDT) [95% CI=5.5-21 month] (p=0.004) **(Figure 3).**

The post-iSPDT median survival (for patients with glioblastoma Grade IV; main group) was 8.2 month [95% CI=5.5-23 month]; it being 5.8 month [95% CI=1.5-12 month] in the control group (without iSPDT) (p=0.012) **(Figure 4).** Survival rates are presented in **Table 6.**

Discussion

Intraoperative sono-photodynamic therapy (iSPDT) is a new promising technique of scientific studies in the field of modern experimental and clinical neuro-oncology.

A key element in the development of irreversible changes in the destructive nature of the tumor are known to be apoptosis, autophagy and necrosis, according to the works devoted to studying the key mechanisms of joint implementation of sonodynamic and photodynamic effects in tumor cells. According to Yumita et al. and Mroz et al. a major role in the development of programmed cell death belongs to sonic processes and photoinitiated oxidation [21,22]. However, sono-photodynamic damage to endothelial cells of capillaries feeding a tumor tissue, leads to the development of vascular stasis, thrombosis and severe cell hypoxia resulting in ischemic necrosis. It should be noted that the factors influencing the development of apoptosis or necrosis are cell type, PS class, its intracellular localization and radiation exposure dose [23].

A number of experimental studies *in vitro* and *in vivo* confirmed the effectiveness of SDT of malignant gliomas using hematoporphyrin, photofrin, radachlorin, rose Bengal, 5-ALA. All trials devoted to the study of the effectiveness of methods and SDT and SPDT of malignant gliomas are experimental [24-31].

In order to prevent thermal damage to normal brain tissue in animals. Jeong et al. suggested the use of ultrasonic fractionated radiation regime with a frequency of 1 MHz and an intensity of 2.65 W/cm^2. 2 weeks after treatment, the volume of tumors in the surgical group was 122.5 ± 39.6 mm^3, in the group of animals treated with ultrasound 87.4 ± 21.4 mm^3, SDT with radachlorin 56.4 ± 12.5 mm^3, SDT with 5-ALA 10.5 ± 8.2 mm^3. The results indicate a high antitumor efficacy of SDT with 5-ALA and radachlorin, as well as the absence of significant side reactions of the treatment, which confirms its safety [27].

Yamaguchi et al. confirmed the sensitivity of gliomas U87-MG to 5-ALA using a low-frequency ultrasonic radiation. Experiments were performed in 20 mice Balb/c, distributed in the following groups: control (n=5), 5-ALA (n=5), US (n=5) and 5-ALA+US (n=5). As the tumor was used subcutaneously transplanted glioma U87-MG. Ultrasonic treatment (25 kHz; 4 min.) was carried through 4 hours after administration of 5-ALA (100 mg/kg). The mean of tumor growth rate of group «5-ALA+US» was 0.08 ± 0.08 (mean ± SE), while the other groups showed high growth rate; intact control − 6.89 ± 1.19 (p=0.0121); 5-ALA − 4.85 ± 1.69 (p=0.0121) and US − 5.08 ± 2.77 (p=0.00214) [28].

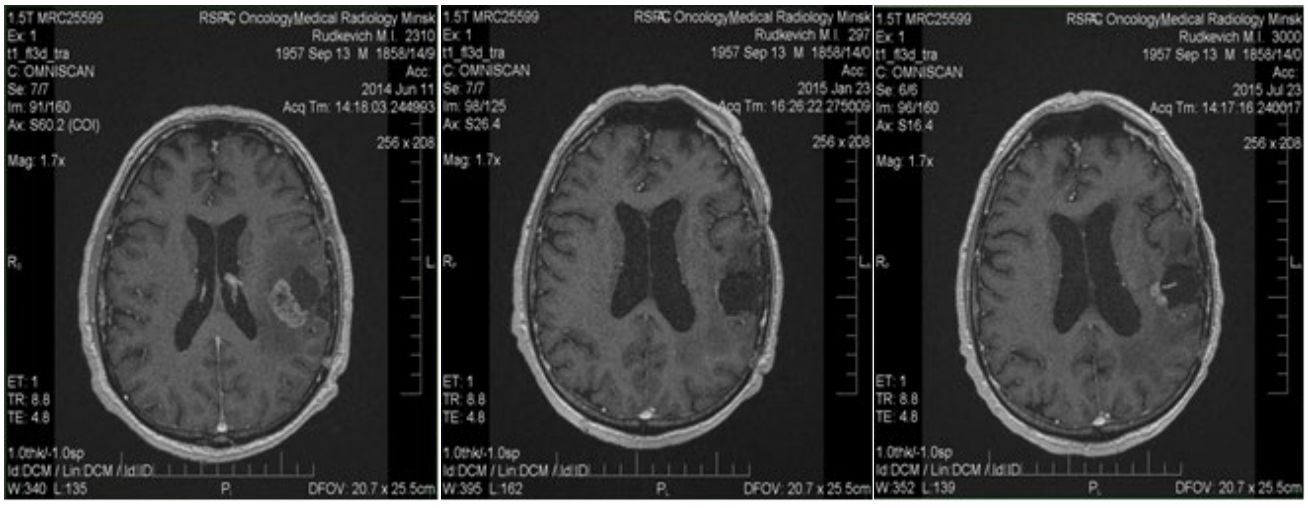

Figure 2 Control follow-up MRI examination from patient with recurrence glioblastoma Grade IV before re-operation (A), 6 months after treatment (B) and 12 months after treatment, including surgical intervention, intraoperative sono-photodynamic therapy and 2 courses of systemic chemotherapy with carmustine (C).

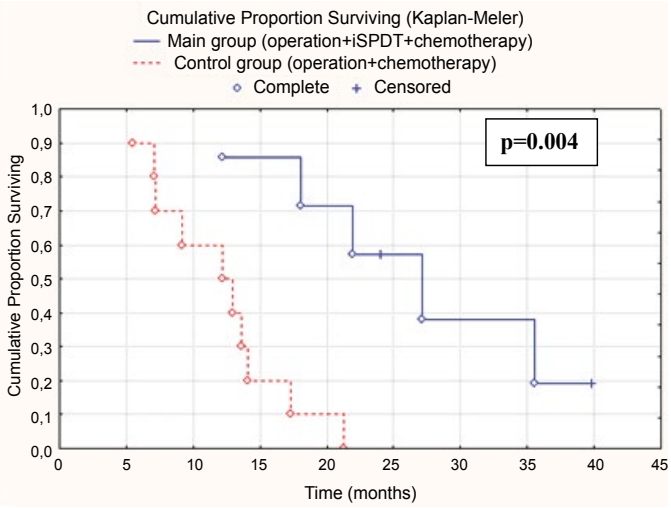

Figure 3 Indicators of overall patients survival in main and control groups.

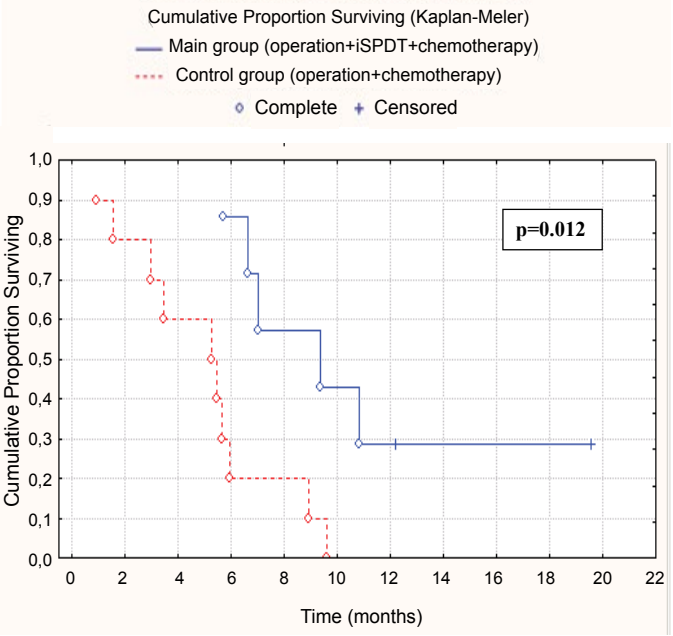

Figure 4 Indicators of patient survival after iSPDT in main and control groups.

Song et al. confirmed the efficiency of SDT (1 MHz; 0.5 W/cm²) with hematoporphyrin monomethyl ether (HMME) at a dose of 10 mg/kg in the treatment of orthotopic C6 glioma in rats on the basis of the change in tumor volume and overall survival of laboratory animals after proposed actions (HMME, US, HMME+US). The control by magnetic resonance imaging (MRI) on 3rd, 7th and 14th days, the first index in the group of PS was significantly less than in the comparison group ($p<0.01$). The medial overall survival of laboratory animals that received treatment with SDT and HMME, exceeded 50 days and was significantly higher than this in the groups with HMME ($p<0.01$) and US ($p<0.01$) [29].

In 2009, a group of Japanese scientists published the first results of the use of SDT with a high-intensity US (25, 110 and 150 W/cm²) and rose Bengal in the experiment *in vivo* (rats). The authors observed a significant inhibition of C6 glioma growth using SDT with an US intensity of 25 W/cm² and PS (3.01 ± 1.74 mm²) compared with untreated control (19.53 ± 3.89 mm²) and a group of animals treated with US alone (10.64 ± 2.21 mm²) [30].

The available literature sources provided sporadic researches on the SPDT application in the treatment of experimental gliomas. Li et al. concluded that the combined use of ultrasonic radiation (0.5 W/cm²; 1 MHz) and PDT (exposure dose <200 J/cm²) with HMME causes marked inhibition of growth of C6 glioma cell (*in vitro*). The authors noted that the death of tumor cells is associated with the implementation of mechanisms of apoptosis witha light exposure dose of 80 J/cm² and above [31].

All experimental *in vivo* studies aimed at studying the antitumor efficacy of SDT and SPDT without prior TR of tumors [27-30]. The hallmark of our *in vivo* trial is to develop a method of treatment of experimental gliomas, including TR with iSPDT.

In our *in vitro* study on C6 glioma cells we showed that photolon produced a pronounced sonosensitizing effect and increased the cytotoxic effect of US by 1.5-2.3-fold [18]. In the *in vivo* study on subcutaneously transplanted glioma C6, most pronounced antitumor effect with the maximal (100%) tumor necrosis was achieved in 2.5 h. after intravenous administration of photolon with ultrasonic exposure at a frequency of 0.88 MHz and pulse intensity of 0.7 W/cm² for 10 minutes, followed by photoirradiation at a dose of 100 J/cm² [19]. In the *in vivo* experiment in rats with orthotopic C6 glioma we demonstrated that median OS significantly higher in laboratory animals treated with TR+iSPDT (38 days), compared with the following groups: TR (18 days; $p=0.001$); vs. TR+iSDT (25 days; $p=0.01$); vs. TR+iPDT (30 days; $p=0.03$) [20].

Table 6 Indicators of patient survival in control and study groups.

Variable	Control group*	Main group**
	value (95% CI)	
Median overall survival (days)	320 (216–423)	632 (436–827)
Overall survival (%)		
At 6 months	88 (70–100)	90 (73–100)
At 12 months	44 (30–58)	80 (60–99)
At 18 months	10 (8–12)	60 (41–78)
At 24 months		30 (21–38)
Median progression-free survival (days)	171 (118–224)	199 (137–260)
Progression free survival (%)		
At 6 months	10 (8–12)	50 (29–58)
At 12 months		10 (8–11)
At 18 months		
At 24 months		

*Control group – Operation+systemic chemotherapy; **Main group – Operation+iSPDT+systemic chemotherapy

Based on these experimental results, we have developed and tested in a pilot clinical protocol, which is based on the study of the safety and efficiency of the iSPDT. According to the first obtained results, iSPDT with photolon is noted to be well tolerated and safe method. Our MRI studies indicate the possibility of stabilization of tumor growth. Median OS and post-iSPDT of patients in main group (23.9 and 8.2 month, respectively) is significantly increased compared to that in the control group (12.1 and 5.8 month; p=0.004 and p=0.012, respectively).

This is the first report to demonstrate the benefits of iSPDT consisting of low-power density US and photoirradiation in clinical neuro-oncology. Successful clinical testing shows the relevance and the prospects for further work within the framework of phase II randomized trial in patients with malignant gliomas.

Conclusions

In our opinion, the combination of different technological solutions allows practitioners to choose an adequate scheme of laser and ultrasonic irradiation of the resected tumor bed of any location and, in the long term, to make the developed method an integral part of the scheme of complex treatment of patients with primary and recurrent forms of malignant gliomas. To determine the antitumor efficacy of the method further research is needed.

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