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EFFECTS OF COMBINED SONODYNAMIC AND PHOTODYNAMIC THERAPIES WITH PHOTOLON ON A GLIOMA C6 TUMOR MODEL

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The *aim* of this study was to investigate the low-power density sonication, sonodynamic therapy (SDT) with Photolon and combination of SDT and photodynamic therapy (PDT) with Photolon for the ablation of glioma C6 tumor model in rats. *Methods:* The study was performed on 50 rats bearing glioma C6. The tumors were sonicated with/without prior intravenous injection of photosensitizer (PS) Photolon (2.5 mg/kg b.w). Sonication was performed with 0.4; 0.7 and 1.0 W/cm² power density at 1 MHz frequency for 10 min, 2.0 h after Photolon administration using BTL-5710 Sono (BTL Industries Limited, Great Britain). PDT was carried out 2.5 h after Photolon administration using diode laser with 661 nm wavelength (IMAF-AXICON, Minsk, Republic of Belarus) at doses of 50 and 100 J/cm² with 0,17 W/cm² fluence rate. Assessment of tumor response was performed by vital staining with Evans blue and pathologic examination. *Results*: The maximal tumor necrosis area that underwent sonication (1 MHz; 0.7 W/cm²; 10 min.) followed by PDT at a dose of 100 J/cm² was 100%. *Conclusion:* This is the first report to demonstrate the benefits of sono-photodynamic therapy (SPDT) consisting of low-power density ultrasound and PDT for the treatment of malignant glioma models.
Key Words: **glioma C6, sonodynamic therapy, photodynamic therapy, Photolon.**

Primary malignant brain tumors account for about 1.5–2% of all human neoplasms and are distinctive of their ability for rapid proliferation, angiogenesis and invasive growth. Gliomas represent the most numerous category (77%) of central nervous system tumors. The principal nosologic forms of gliomas are astrocytomas (anaplastic astrocytoma, glioblastoma multiforme), oligodendrogliomas, ependimomas and mixed gliomas [1].

The conventional regimens of glioma therapy involve surgical intervention, radiotherapy, chemotherapy, immune correction therapy and specific antitumor immunotherapy. Nevertheless, despite the evident accomplishments of medical science over the past decades, the management of these patients largely remains an unsettled issue of current clinical neurooncology. The techniques used for the time being improve, at the best, their mean survival and prolong the recurrence-free period. The administered treatment is associated with high postoperative mortality rates and the risk of severe morbidity development.

An active search is currently underway for new alternative treatment techniques for malignant glial brain tumors. One of such techniques is sonodynamic therapy (SDT). SDT is based on a significant increase in drug cytotoxicity in the focal lesion exposed to ultrasound of 0.5–3.0 MHz frequency and 0.5–5.0 W/cm² power density, resulting in cell destruction and killing [2–4]. The substances acquiring cancericidal abilities when affected by ultrasound are acknowledged as sonosensitizers. Radiosensitizers (dimexide, metronidazole) and some chemotherapeutic agents (carboplatin, methotrexate and othes) are assigned to the sonosensitizer class in the first instance.

However, in the early 90s of the 20th century, two independent research groups (S. Umemura et al., 1990; D. Kessel et al., 1994) reported their first results demonstrating the high efficacy of SDT with photosensitizers (PS) of the porphyrin series [5, 6]. At present, several theories are available, explaining the mechanisms underlying SDT. A number of authors hypothesized that PS sonomodification was based on photochemical reactions. However, ultrasound radiation of certain parameters is the trigger mechanism of SDT rather than a light quantum [7]. According to V. Misik, the main acting element in its implementation is the phenomenon of cavitation realized on cellular and subcellular levels [8].

The data of numerous studies have been currently published abroad, concerning the use of a number of 1st and 2nd generation PS (hematoporphyrin, photofrin I and II, meso- and protoporphyrin IX, ATX-70, pheophorbide-A, ALPcS4, chlorine PAD-S31 and e6, methylene blue, Rose Bengal, 5-ALA and others). The results of *in vitro* and *in vivo* trials suggest that SDT is a promising treatment modality for such malignant tumors as breast, lung, hepatic, intestinal and renal cell cancers, soft tissue sarcoma, ascetic forms of ovarian neoplasms, leukemias [9–13].

Over the recent years, SDT aroused certain interest as a novel and highly potential treatment modality for malignant gliomas. A number of experimental model studies obtained reassuring results of employing this modality in the treatment of different glial tumor strains with hematoporphyrin and Rose Bengal [14–16]. At the same time, a noticeable trend in research is combined employment of photodynamic therapy (PDT) and SDT in the management of glial tumors.

The primary purpose of this study is to investigate the antitumor effect of SDT and PDT with Photolon on a glioma C6 tumor model in white random-bred rats.

MATERIALS AND METHODS

Experimental animals and tumors. Fifty white random-bred rats obtained from the vivarium of the N.N. Alexandrov National Cancer Center of Belarus

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Abbreviations used: PDT – photodynamic therapy; SDT – sonodynamic therapy; SPDT – sono-photodynamic therapy.

(Minsk, Republic of Belarus) were used. The animals received a standard diet and had permanent access to water. Experimental rat glioma C6 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 implanted in a serial in mode. For experiments, tumor homogenate was implanted subcutaneously into the inguinal area by the injection of 0.5 ml of 10% tumor cells suspension in Hanks' solution. The experiments were performed 11–14 days after tumor implantation. Before the treatment the animals were anesthetized with droperidol (5.0 mg/kg) and fentanil (0.05 mg/kg) and immobilized. 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 "Guide lines for experimental (preclinical) studies of new pharmacologic substances"(Health Ministry of Russian Federation, State Pharmacologic Committee of Russian Federation, Moscow, 2000).

PS. Chlorin e6 conjugated with polyvinyl pyrrolidone (Photolon® produced by Scientific Pharmaceutical Center of RUE "Belmedpreparaty", Minsk, Republic of Belarus) was injected in the tail vein at standard dose of 2.5 mg/kg.

PDT. Photoirradiation of tumors was carried out 2.5 h after Photolon administration using diode laser with 661 nm wavelength (IMAF-AXICON, Minsk, Republic of Belarus) at doses of 50 and 100 J/cm^2 with 0.17 W/cm^2 fluence rate. The output was 0.3 W, the light spot diameter 1.5 cm, irradiation for 5 and 10 min.

SDT. Tumor insonation procedure was performed 2.0 h after Photolon administration using BTL-5710 Sono (BTL Industries Limited, Great Britain) with an emitter of 5.0 cm^2, 1 MHz ultrasound frequency in a continuous mode with 0.4; 0.7 and 1.0 W/cm^2 intensity for 10 min employing stable techniques.

Sono-photodynamic therapy (SPDT). Tumor insonation procedure was performed 2.0 h after Photolon administration with 1 MHz ultrasound frequency in a continuous mode with 0.4; 0.7 and 1.0 W/cm^2 power density for 10 min. Photoirradiation of tumors was delivered at doses of 50 and 100 J/cm^2 with 0.17 W/cm^2 fluence rate. The output was 0.3 W, the light spot diameter 1.5 cm, irradiation for 5 and 10 min.

Antitumor efficacy of PDT/SDT with Photolon was evaluated 24 h after the treatment by quantification of glioma C6 tumor necrosis area by vital staining of tumor bearing animals with 0.6% Evans' blue solution. The animals were sacrificed by chloroform; the tumors were removed, fixed in 10% formalin solution and frozen. Transverse tumor sections 2–3 mm thick were made. Necrotic tumor areas due to direct effect on tumor cells or structural and functional disorders in microcirculation remained unstained. The percentage of tumor necrotic unstained parts was evaluated using "ImageJ" (NIH, Bethesda, USA).

Statistical analysis. The values obtained were processed using standard statistical methods of Origin Stat 7.0 software. The significance level was determined as 0.05.

RESULTS AND DISCUSSION

This study has made a comparative evaluation of antitumor efficacy of low power density ultrasound, SDT, PDT and their combination with prior i.v. introduction of Photolon. Local ultrasound treatment (1 MHz, 10 min) of the glioma C6 rat tumor model with 0.4, 0.7 and 1.0 W/cm^2 power density without prior PS administration caused tumor necrosis (44.62 ± 10.17%, 53.54 ± 5.23% and 66.27 ± 6.65% respectively) (Table 1, Fig. 1).

Table 1. Necrosis area in histotopographic sections of glioma C6 rat tumor after ultrasound treatment with power density 0.4; 0.7 and 1.0 W/cm^2

Groups	Number of sections	Tumor area, cm^2	Necrosis area	
			cm^2	%
Ultrasound 0.4 W/cm^2	12	3.67±1.73	1.64±0.34	44.62±10.17
Ultrasound 0.7 W/cm^2	14	1.98±0.43	1.06±0.23	53.54±5.23
Ultrasound 1.0 W/cm^2	12	2.42±0.27	1.61±0.32	66.27±6.65

Note: *$p < 0.05$

Table 2 and Fig. 2 present necrosis areas on histotopographic sections of glioma C6 after SDT (1 MHz, 10 min) with 0.4, 0.7 and 1.0 W/cm^2 power density, ultrasound treatment being performed 2 hours after Photolon administration at a dose of 2.5 mg/kg. The percentage of tumor necrosis areas was 61.04 ± 4.77%, 82.65 ± 2.41% and 79.71 ± 4.66% respectively).

Table 2. Necrosis area in histotopographic sections of glioma C6 rat tumor after i.v. injection of Photolon at a dose of 2.5 mg/kg and ultrasound irradiation with power density 0.4; 0.7 and 1.0 W/cm^2

Groups	Number of sections	Tumor area, cm^2	Necrosis area	
			cm^2	%
Photolon + Ultrasound 0.4 W/cm^2	11	2.92±0.21	1.78±0.13	61.04±4.77
Photolon + Ultrasound 0.7 W/cm^2	12	2.78±0.52	2.21±0.27	82.65±2.41
Photolon + Ultrasound 1.0 W/cm^2	13	4.37±0.83	3.48±0.35	79.71±4.66

Note: *$p < 0.05$

A significant increase in the values under study was noted in rats treated with SDT vs controls (ultrasound therapy without prior PS administration) ($p < 0.05$).

Table 3 and Fig. 3 demostrate necrosis areas on histotopographic sections of glioma C6 after PDT at light exposure doses of 50 and 100 J/cm^2 (0.17 W/ cm^2 power density). The percentage of tumor necrosis areas was 61.42 ± 2.62% and 85.52 ± 3.79% respectively.

Table 3. Necrosis area in histotopographic sections of glioma C6 rat tumor after i.v. injection of Photolon at a dose of 2.5 mg/kg and photoirradiation at doses of 50 and 100 J/cm^2

Groups	Number of sections	Tumor area, cm^2	Necrosis area	
			cm^2	%
Photolon + Photoirradiation 50 J/cm^2	10	3.46±0.21	2.13±0.17	61.42±2.62
Photolon + Photoirradiation 100 J/cm^2	12	4.01±0.19	3.43±0.16	85.52±3.79

Note: *$p < 0.05$

On 22 histotopographic sections, Table 4 and Fig. 4 present the results of combination treatment using local ultrasound radiation with 0.7 W/cm^2 power density and local photoirradiation of tumors at 50 and 100 J/cm^2 light exposure doses respectively.

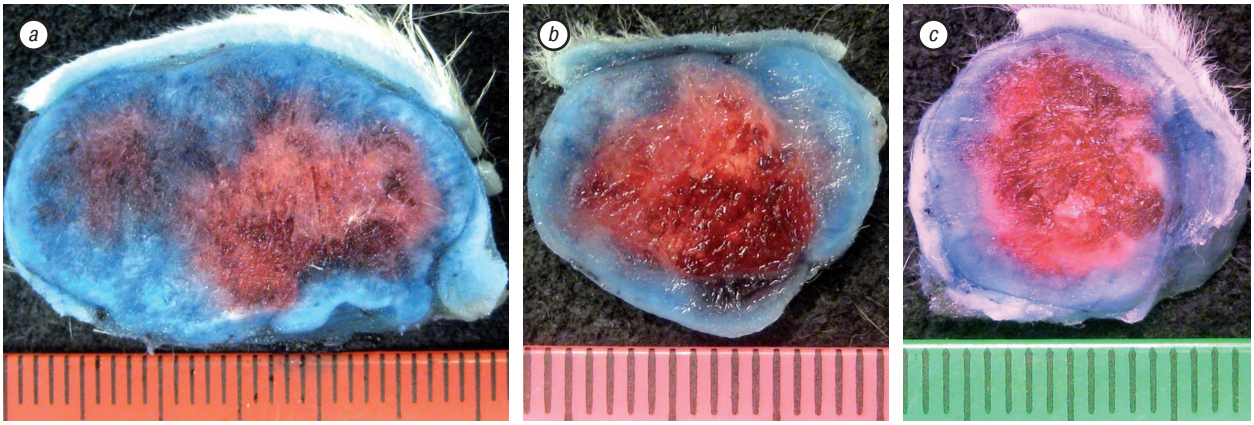

Fig. 1. Histotopographic sections of glioma C6 rat tumor after ultrasound treatment with power density 0.4 (*a*); 0.7 (*b*) and 1.0 (*c*) W/cm^2

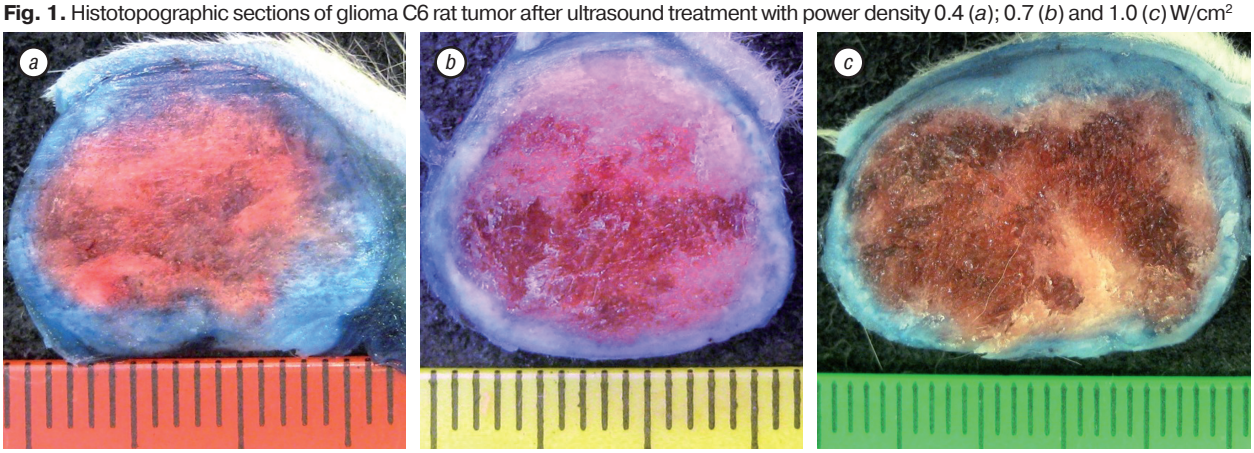

Fig. 2. Histotopographic sections of glioma C6 rat tumor after SDT with power density 0.4 (*a*); 0.7 (*b*) and 1.0 (*c*) W/cm^2

Table 4. Histotopographic sections of glioma C6 rat tumor after SDT with power density 0.7 W/cm^2 and PDT at doses of 50 (*a*) and 100 (*b*) J/cm^2

Groups	Number of sections	Tumor area, cm^2	Necrosis area	
			cm^2	%
Photolon + Ultrasound 0.7 W/cm^2 + Photoirradiation 50 J/cm^2	10	3.87±0.14	3.31±0.18	85.64±5.33
Photolon + Ultrasound 0.7 W/cm^2 + Photoirradiation 100 J/cm^2	12	4.33±0.16	4.33±0	100

Note: *$p < 0.05$

The results obtained suggest that combination treatment including SDT and PDT of certain parameters enhances the effect on the glial tumor model in rat brain. The combination treatment significally (by 25–30%) increases necrosis areas in tumor tissues compared with each of the components taken separately. The optimal therapy regimen involves local 1 MHz frequency ultrasound with 0.7 W/cm^2 power density and photoirradiation at a light exposure dose of 100 J/cm^2 after prior i.v. Photolon administration at a dose of 2.5 mg/kg.

SDT for malignant glial tumors is a novel and promising trend in neurooncology. At the given stage of its formation the profile of this treatment modality is experimental. A number of *in vivo* studies reported promising results of its use in the management of rat gliomas with such sonosensitizers as hematoporphyrin, Rose Bengal and others [15, 16].

Our trial of laboratory rats with glioma C6 has reaffirmed sonosensitizing activity of Photolon, thus being a drug of prospective advantages for SDT of glial brain tumors. We have found evidence suggesting that the

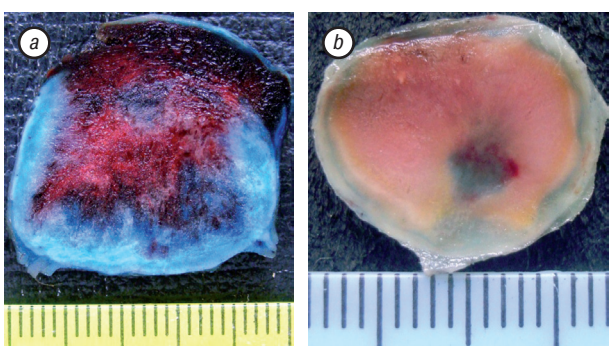

Fig. 3. Histotopographic sections of glioma C6 rat tumor after PDT at doses of 50 (*a*) and 100 (*b*) J/cm^2

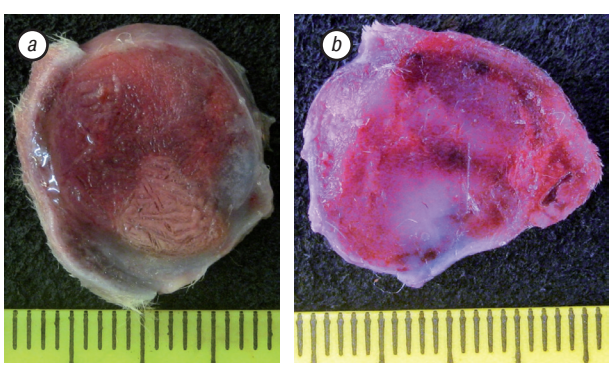

Fig. 4. Histotopographic sections of glioma C6 rat tumor after SDT with power density 0.7 W/cm^2 and PDT at doses of 50 (*a*) and 100 (*b*) J/cm^2

combined employment of ultrasound and the photosensitizing agent leads to strong damage of tumor tissue as a result of developing induced chemical reactions and cavitation effect implementation in the

tumor cell. The use of low power density mode of ultrasound treatment is safe, and possible application in the clinical setting would not be associated with high risk of thermal damage of normal brain tissues.

A promising lead for further research is evaluation of antitumor efficacy of combined ultrasound and laser radiation treatment in the management of glial brain tumors (SPDT). Our encouraging results and the few reports in this field allow to define the SPDT modality as a growing trend in current neurooncology.

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