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 at 0.4; 0.7 and 1.0 W/cm² power density at 1 MHz frequency for 20 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.

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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 fentanyl (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/cm² with 0.17 W/cm² 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 cm², 1 MHz ultrasound frequency in a continuous mode with 0.4; 0.7 and 1.0 W/cm² 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/cm² power density for 10 min. Photoirradiation of tumors was delivered at doses of 50 and 100 J/cm² with 0.17 W/cm² 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, PDT, 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/cm² 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/cm².**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of sections</th>
<th>Tumor area, cm²</th>
<th>Necrosis area cm²</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ultrasound 0.4 W/cm²</td>
<td>12</td>
<td>3.67±1.73</td>
<td>1.64±0.34</td>
<td>44.62±10.17</td>
</tr>
<tr>
<td>Ultrasound 0.7 W/cm²</td>
<td>14</td>
<td>1.98±0.43</td>
<td>1.06±0.23</td>
<td>53.54±5.23</td>
</tr>
<tr>
<td>Ultrasound 1.0 W/cm²</td>
<td>12</td>
<td>2.42±0.27</td>
<td>1.81±0.32</td>
<td>66.27±6.65</td>
</tr>
</tbody>
</table>

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/cm² 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/cm².**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of sections</th>
<th>Tumor area, cm²</th>
<th>Necrosis area cm²</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photolon + Ultrasound</td>
<td>11</td>
<td>2.92±0.21</td>
<td>1.78±0.13</td>
<td>61.04±4.77</td>
</tr>
<tr>
<td>Photolon + Ultrasound</td>
<td>12</td>
<td>2.78±0.52</td>
<td>2.21±0.27</td>
<td>82.65±2.41</td>
</tr>
<tr>
<td>Photolon + Ultrasound</td>
<td>13</td>
<td>4.37±0.83</td>
<td>3.48±0.35</td>
<td>79.71±4.66</td>
</tr>
</tbody>
</table>

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 demonstrate necrosis areas on histotopographic sections of glioma C6 after PDT at light exposure doses of 50 and 100 J/cm² (0.17 W/cm² 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/cm².**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of sections</th>
<th>Tumor area, cm²</th>
<th>Necrosis area cm²</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photolon + Photoirradia-</td>
<td>10</td>
<td>3.46±0.21</td>
<td>2.13±0.17</td>
<td>61.42±2.62</td>
</tr>
<tr>
<td>tion 50 J/cm²</td>
<td>Photolon + Photoirradia-</td>
<td>12</td>
<td>4.01±0.19</td>
<td>3.43±0.16</td>
</tr>
<tr>
<td>tion 100 J/cm²</td>
<td>Photolon + Photoirradia-</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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/cm² power density and local photoirradiation of tumors at 50 and 100 J/cm² light exposure doses respectively.
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$^2$

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of sections</th>
<th>Tumor area, cm$^2$</th>
<th>Necrosis area, cm$^2$</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photolon + Ultrasound 0.7 W/cm$^2$ + Photoirradiation 50 J/cm$^2$</td>
<td>10</td>
<td>3.87±0.14</td>
<td>3.31±0.18</td>
<td>85.64±5.33</td>
</tr>
<tr>
<td>Photolon + Ultrasound 0.7 W/cm$^2$ + Photoirradiation 100 J/cm$^2$</td>
<td>12</td>
<td>4.33±0.16</td>
<td>4.33±0</td>
<td>100</td>
</tr>
</tbody>
</table>

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 significantly (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 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.

REFERENCES