Experimental Study of Combined Therapy for Malignant Glioma


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The efficiency of photodynamic therapy with photosensitizer Tiosens (Russia) was evaluated in mono- and combined therapy of rats with malignant gliomas (glioblastoma 101/8, oligodendroglioma 14-4-9, and C6 glioma). The efficiency of photodynamic monotherapy was not high: the animals died from brain edema developing in tumor tissue and in the adjacent normal cerebral tissue. Pathomorphological studies of tumor tissue detected necrosis and apoptosis, destruction of vessels with hemorrhages, and vascular thrombosis. Combined therapy for malignant gliomas including Tiosens photodynamic therapy and subsequent temodal or lysomustine chemotherapy, was the most effective. In glioblastoma 101/8, combined therapy with lysomustine or temodal led to prolongation of the lifespan by 127%; 62.5 and 50% rats were cured, respectively; in oligodendroglioma 14-4-9, animal lifespan was prolonged by 80 and 60%, with 43 and 45% rats cured, respectively. Glioma C6 was least sensitive to therapy.

Key Words: rat malignant glioma; photodynamic therapy; Tiosens; chemotherapy; combined therapy

Primary tumors of the brain are responsible for just 1-2% of cancer-related morbidity. Therapy for tumors of this location is difficult, because the tumor develops in the CNS that is protected by the blood-brain barrier. On the one hand, this prevents extracranial metastases, on the other hand, impedes systemic treatment for these tumors [4]. Despite the efforts of scientists and clinicians, no appreciable improvement has been attained in the treatment of this cohort of patients over the recent decades. The mean lifespan of patients after combined therapy is 8-12 months; 5-year survival is no higher than 4-7%. Importantly, these values are the lowest in practical oncology.

Optimization of the results of combined therapy for malignant gliomas implies the search for new effective antitumor drugs and the use of new rational therapeutic methods. One of these methods is photodynamic therapy (PDT). The important advantage of PDT is the absence of grave local and systemic complications [2].

The efficiency of photodynamic destruction of sensitized cells is determined by the intracellular concentration of the sensitizer, its location in the cells and photochemical activity, and by the laser photodose delivered to the tumor. In addition to the direct toxic effect of PDT on tumor cells, blood supply disorders make an important contribution to the destruction of the malignant tumor, due to damage to the vascular endothelium in the tumor tissue and cytokine reactions [1].
In *vitro* and *in vivo* experiments and clinical studies confirmed the efficiency of PDT with photosensitizers photolon, alasense, photofrin, hematoporphyrin, foscan, etc., in brain tumors. The survival median after intra- and postoperative PDT reaches 21 months [3].

An important approach to improving the efficiency of PDT is the search for photosensitizers with absorption at 700-800 nm (which correspond to minimum absorption of biological tissues). The use of these photosensitizers minimizes the loss related to absorption by the tissue, increases the depth of light penetration, and improves the therapeutic efficiency [5].

We evaluated the efficiency of PDT with Tiosens, a Russian photosensitizer, in combined therapy for gliomas on three tumor models on rats (C6 glioma, 101/8 glioblastoma, and 14-4-9 oligodendroglioma).

**MATERIALS AND METHODS**

The study was carried out on adult outbred and Wistar rats (200-220 g).

Three tumor models were used: glioblastoma 101/8 with a stable structure of isomorphic malignant glioblastoma; C6 glioma classified by histological structure as anaplastic astrocytoma and resembling human glioma; and oligodendroglioma 14-4-9. The tumors were transplanted intracranially into the bottom of the right cerebral lateral ventricle. The transplantation efficiency was 95-98%.

Tiosens (hydroxyaluminium tetra-3-phenylthiophthalocyanin) insoluble in water was synthesized at Research Institute of Organic Semiproduits and Stains. The liposomal dosage form of Tiosens developed at the Laboratory of Dosage Forms of N. N. Blokhin Russian Cancer Research Center was injected intravenously (3 mg/kg) 6 days after tumor transplantation and 24 h before PDT. The electron spectrum of Tiosens absorption had peaks at 717±4, 648±4, 450±3, and 342±3 nm.

Tiosens accumulation was evaluated by the spectral fluorescent method on a LESA-01-BIOSPEC spectromonitor. Tiosens fluorescence was excited with LFD-730-01-BIOSPEC laser at λ=720 nm. Laser exposure was carried out in doses of 120 and 60 J/cm².

Lysomustine was injected in a single intravenous dose of 80 mg/kg 3 h after laser exposure. Temodal was administered orally at 48-h interval (total dose 50 mg/kg). The first temodal dose was administered 3 h after laser exposure. Pathomorphological studies were carried out by the standard method on preparations stained with hematoxylin and eosin.

Lifespan prolongation (LSP; %) of experimental rats in comparison with controls (evaluated by Fisher’s and Student’s test) and pathomorphological findings served as the criteria of antitumor effect.

**RESULTS**

In order to determine the optimal terms for PDT after tumor transplantation, we evaluated the level and selectiveness of Tiosens accumulation in glioma tissue after its intravenous injection (3 mg/kg) by the spectral fluorescent method. Laser exposure was the most effective on day 6 after tumor transplantation and 24 h after Tiosens injection.

Analysis of rat survival after Tiosens (3 mg/kg) PDT through a trephination hole in the optimal laser exposure mode (100 mW/5 mm², 20 min, 120 J/cm²) showed a 42% LSP in rats with C6 glioma and 30% LSP in oligodendroglioma 14-4-9. The efficiency of Tiosens PDT in glioblastoma 101/8 was evaluated after interstitial laser exposure in a dose of 54 J/cm². A special lightguide was inserted into the cranial cavity to a depth of 1-2 mm in order to reduce energy absorption by surface tissues (skin and bone). The results indicated a 22% LSP. The main cause of poor efficiency of Tiosens PDT monotherapy was marked damage to all layers of the tumor tissue and of adjacent normal cerebral tissue in interstitial exposure, which led to release of inflammation mediators and manifest cerebral edema.

Pathomorphological studies of the tumor tissue after Tiosens PDT showed necrosis and apoptosis, destruction of vessels with hemorrhages and thrombosis as a result of destructive vasculitis. Necrosis, edema, and polymorphonuclear infiltration were found in the adjacent tissue; proliferation of the glia and endotheliocytes, acute inflammatory reaction, and reparative changes in the nerve tissue were detected.

In order to improve the efficiency of Tiosens PDT in brain tumors, we used it in combination with chemotherapy.

Chemotherapy was carried out with lysomustine inhibiting DNA synthesis in tumor cells by 80% (mainly during the last third of the S phase) 48-96 h after injection. In previous studies, this drug exhibited

### Table 1. Efficiency of Lysomustine and Temodal Monotherapy in Rat Gliomas

<table>
<thead>
<tr>
<th>Tumor model</th>
<th>Drug, dose, mg/kg</th>
<th>Cure, %</th>
<th>LSP, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glioma C6</td>
<td>Lysomustine, 80</td>
<td>–</td>
<td>26</td>
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<td>Temodal, 50</td>
<td>–</td>
<td>68</td>
</tr>
<tr>
<td>Oligodendro-glioma 14-4-9</td>
<td>Lysomustine, 80</td>
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<tr>
<td></td>
<td>Temodal, 50</td>
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<tr>
<td>Glioblastoma 101/8</td>
<td>Lysomustine, 80</td>
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<td>83</td>
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<td></td>
<td>Temodal, 50</td>
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<td>54</td>
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