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STUDY OF THE ANTITUMOR ACTIVITY OF 2-(1,1-DIMETHYL-1H-BENZO[E]INDOLIN-2-YL)-5,6,7-TRICHLORO-1,3-TROPOLONE IN COMBINATION WITH TEMOZOLOMIDE ON THE U87 GLIOBLASTOMA MODEL IN VIVO

Glioblastoma remains one of the most aggressive and treatment-resistant brain tumors, highlighting the need for novel therapeutic approaches. This study aimed to evaluate the antitumor efficacy of 2-(1,1-dimethyl-1H-benzo[e]indolin-2-yl)-5,6,7-trichloro-1,3-tropolone in combination with temozolomide (TMZ) in subcutaneous xenografts of U87 glioblastoma cells in Balb/c Nude mouse line. The animals were divided into three groups: (1) TMZ monotherapy (20 mg/kg), (2) combination therapy with tropolone and TMZ (20 mg/kg each), and (3) an untreated control group. Tumor volumes were measured three times weekly, and tumor growth inhibition (TGI%) along with relative tumor mass were calculated at the end of the experiment. On day 25, the mean tumor volumes were as follows: 443.02±52.16 mm³ in the monotherapy group, 395.80±41.98 mm³ in the combination therapy group, and 1331.43±65.65 mm³ in the control group. The results demonstrated that the combination of tropolone and temozolomide significantly suppressed tumor growth compared to the control group. Furthermore, the tumor volume in the combination therapy group was smaller than in the TMZ monotherapy group, although the difference was not statistically significant. The findings revealed that combination therapy resulted in a greater reduction in tumor volume and relative tumor mass compared to TMZ monotherapy, with TGI rates of 70.27% versus 66.72%, respectively. Histological analysis confirmed marked cellular dystrophic changes in tumors subjected to combination therapy. These results suggest a potential synergistic interaction between tropolone and temozolomide, which may enhance the efficacy of glioblastoma treatment.

Keywords: glioblastoma, U87 cell line, tropolone, temozolomide, immunodeficient mice, antitumor activity, chemotherapy.

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Introduction. Glioblastoma is the most aggressive and lethal form of primary brain tumor. It is characterized by rapid growth, diffuse infiltration, and resistance to conventional therapeutic interventions. Despite strides in surgical techniques, radiation therapy, and chemotherapy, the prognosis for patients remains dismal, with a median survival of a mere 14.6 months [15]. Temozolomide (TMZ), a standard alkylating drug in the treatment of glioblastoma, has seen its efficacy diminished by resistance and adverse effects, underscoring the need for novel therapeutic approaches [15].

Several studies have investigated combination strategies with various compounds to enhance the action of TMZ, including histone deacetylase (HDACis) inhibitors and PI3K/AKT/mTOR signaling pathways, as well as natural compounds such as curcumin and resveratrol. These approaches show synergy with TMZ, enhancing its pro-apoptotic action and increasing tumor cell sensitivity [9].

Tropolones, low-molecular compounds with a unique structure and pronounced antitumor properties, attract particular attention. They inhibit key enzymes such as MMP and HDAC and are able to induce apoptosis, inhibit angiogenesis, and regulate the levels of reactive oxygen species in tumor cells. Some tropolones derivatives exhibit antiproliferative and proapoptotic effects, making them promising for the treatment of glioblastoma in combination with TMZ [2, 4–6].

Purpose of the study – to evaluate the antitumor activity of 2-(1,1-dimethyl-1H-benzo[e]indolin-2-yl)-5,6,7-trichloro-1,3-tropolone in combination with temozolomide against subcutaneous xenografts of U87 glioblastoma cell culture in immunodeficient Balb/c Nude mice.

Materials and methods. In the present experiment, 18 female Balb/c Nude mice were utilized. These mice were

maintained in a pathogen-free environment with unlimited access to food and water. The mice in all of the studied groups were weighed at the beginning and at the conclusion of the experiment. All experimental procedures were carried out in accordance with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Scientific Purposes (ETS 123, Strasbourg, March 18, 1986) and were approved by the Biotic Commission of the National Medical Research Centre for Oncology of the Ministry of Health of Russia.

Human glioblastoma cell line U87 was cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a humidified atmosphere containing 5% CO2. U87 cells (5 × 10^6) were injected subcutaneously into the right flank of each mouse in a mixture of serum-free DMEM and Matrigel Matrix.

The studied tropolone derivative, 2-(1,1-dimethyl-1H-benzo[e]indolin-2-yl)-5,6,7-trichloro-1,3-tropolone (JO-122(2)), synthesized at the SFEDU Research Institute of Physical Chemistry, was administered orally 3 times a week for 3 weeks [5]. Temozolomide (TMZ) was dissolved in 0.9% NaCl solution and administered intraperitoneally daily for 3 weeks. The drugs were administered regardless of food and water intake.

After the tumors reached an average volume of ~100 mm³, the mice were divided into three groups (n = 6), with the average volume of subcutaneous xenografts between the groups not differing by more than 10%: group 1 – TMZ (20 mg/kg), group 2 – a combination of TMZ and tropolone (20 mg/kg each), group 3 – control group.

Tumor growth was measured every three days, starting with the first administration of the drugs. Tumor volume was calculated using the formula:

$$V = LW^2/2,$$

where V is the tumor volume (mm³), L and W are the length and width of the tumor (mm).

To assess antitumor activity, the tumor growth inhibition index (TGI%) was used:

$$TGI\ (\%) = (Vk - Vo) / Vk \times 100,$$

where Vk and Vo are the average tumor volume (mm3) in the control and experimental groups, respectively.

The experiment lasted 25 days. The euthanasia procedure was performed by decapitation, after which the tumor material was extracted. The tumors were weighed to calculate the relative mass using the formula:

$$m_{rel}\ (\%) = (m_t / m_b) \times 100$$

where m_t is the tumor mass (g), m_b is the animal's body mass (g).

For histological analysis, the obtained tumor material was stained with hematoxylin and eosin.

Data are presented as mean ± standard error of the mean (SEM). Statistical analysis of data was performed using Statistica 10. Comparisons between groups were performed using one-way analysis of variance (ANOVA). A p value of <0.05 was considered statistically significant.

Research results and discussion. The experiment assessed the effect of temozolomide and its combination with the studied tropolone on the growth of glioblastoma tumors. The results showed a significant effect on tumor growth compared to the control group (Figure 1).

On the 7th day of the experiment, a statistically significant decrease in tumor volume was noted in the experimental groups compared to the control group. By the end of the 25-day period of drug administration, the average tumor volume in the group receiving the combination of tropolone and TMZ (group 2) was 395.80±41.98 mm³. This value was lower than in the TMZ monotherapy group (group 1443.02±52.16 mm³) and the control group (group 3, 1331.43±65.65 mm³).

In the TMZ monotherapy group, tumor growth was slower than in the control group, confirming the antitumor activity of the drug. In combination therapy, a more pronounced effect was noted, suggesting a synergistic or additive mechanism. However, no statistically significant differences were found between the monotherapy and combination groups, indicating similar efficacy of the approaches.

TMZ therapy at a dose of 20 mg/kg significantly reduced the relative tumor mass compared to the control group (Figure 2). In Group 1, the relative tumor mass was 1.12±0.30%, and the TGI index reached 66.72%, indicating a pronounced antitumor effect of the drug. Figure 3 shows tumor nodes of mice from the experimental and control groups, which allows for a visual assessment of the sample sizes.

In the combination therapy group, the relative tumor mass decreased to 0.95±0.17%, and TGI was 70.27%. This confirms the more pronounced effect of combination therapy, possibly due to the synergistic effect of the interaction of tropolone and TMZ.

After completion of therapy, tumor nodes were subjected to histological analysis (Figure 4).

Histological analysis of the specimen revealed a malignant tumor, the structural characteristics of which are consistent with those of glioblastoma. The presence of minor foci of necrosis was observed in the relevant fields of view. Furthermore, dystrophic changes of individual tumor cells were observed in tumor samples exposed to temozolomide. Concomitant exposure to both temozolomide and the other agent resulted in more pronounced dystrophic changes in cells and signs of karyopyknosis in

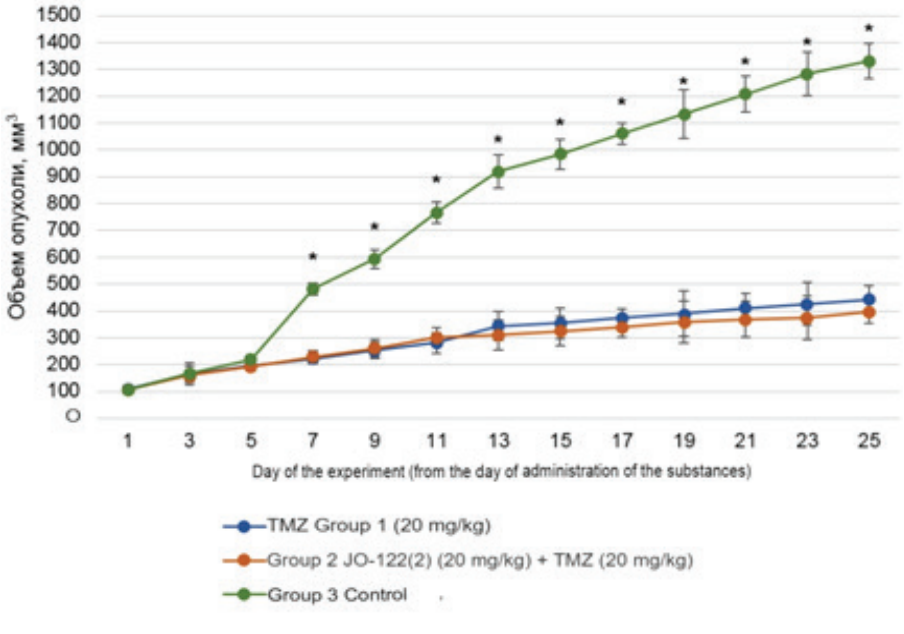

Fig. 1. Average tumor node volumes in Balb/c Nude mice over time. * – statistically significant differences from groups 1 and 2 (p<0.05).

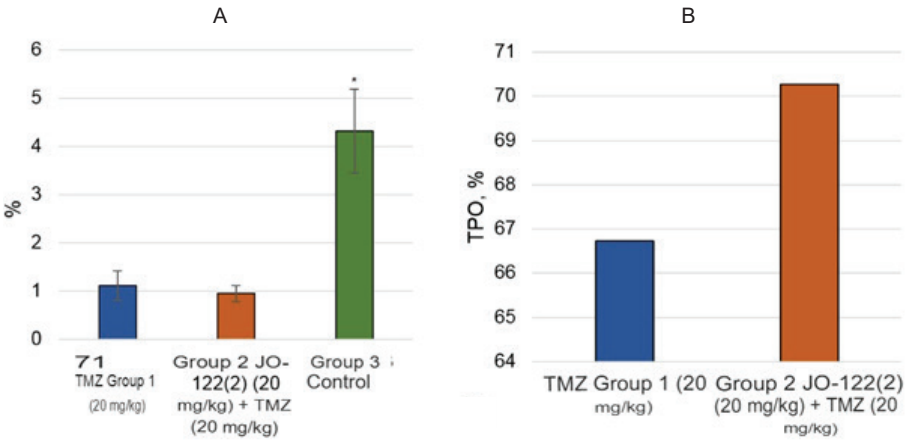

Fig. 2. Relative tumor mass (A) and tumor growth inhibition (TGI) (B) indices in the experimental and control groups

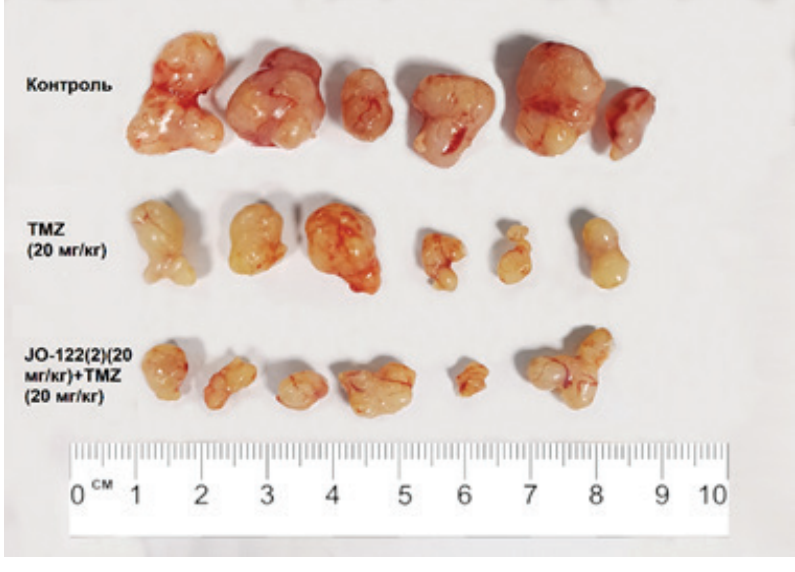

Fig. 3. Tumor nodes of mice from the experimental and control groups

individual tumor nuclei. These findings may indicate induction of cell death due to the combined therapy.

The analysis showed that the administration of TMZ and the combination with tropolone was well tolerated: the body weight and condition of the animals remained stable, and there were no signs of toxicity.

The results of the study demonstrate the potential of the new tropolone derivative as a therapeutic agent for the treatment of glioblastoma. In particular, its combination with temozolomide resulted in an increase in the TGI index, a decrease in tumor weight and characteristic histological changes. These data indicate a possible interaction between tropolone and temozolomide, which may improve therapeutic results and possibly allow a reduction in the dose of temozolomide to reduce its side effects.

It is noteworthy that the dose of temozolomide (TMZ) used in this study was 20 mg/kg (60 mg/m² [12]), which is lower than the standard clinical dose of 75–100 mg/m² [15]. Such a reduction may significantly reduce the severity of side effects of the drug.

Tropolone derivatives are known to have high cytotoxic activity. For example, one of the tropolones showed a pronounced cytotoxic effect on gastric adenocarcinoma (AGS) cells, surpassing fluorouracil (5-FU) even at lower concentrations [4]. In addition, it demonstrated significant efficacy against primary glioma cell cultures [3] and was almost ten times more effective than 5-FU on the SW620 cell line [1]. Its antiproliferative effect on A431 epidermal carcinoma cells was also revealed [7].

The mechanisms underlying the proposed synergy between tropolone and temozolomide require further study. Tropolones are thought to induce caspase-dependent apoptosis and suppress antiapoptotic proteins (e.g., Bcl-2), whereas temozolomide induces apoptosis via DNA damage. Co-administration of the drugs may also enhance p53-dependent apoptosis [2,11,14].

Tropolones also inhibit the Wnt/β-catenin pathway, limiting tumor cell proliferation and migration, and increase reactive oxygen species, causing DNA damage. Temozolomide complements these effects by enhancing oxidative stress [8, 10–11]. In addition, tropolones increase endoplasmic reticulum stress, promoting cell death [13].

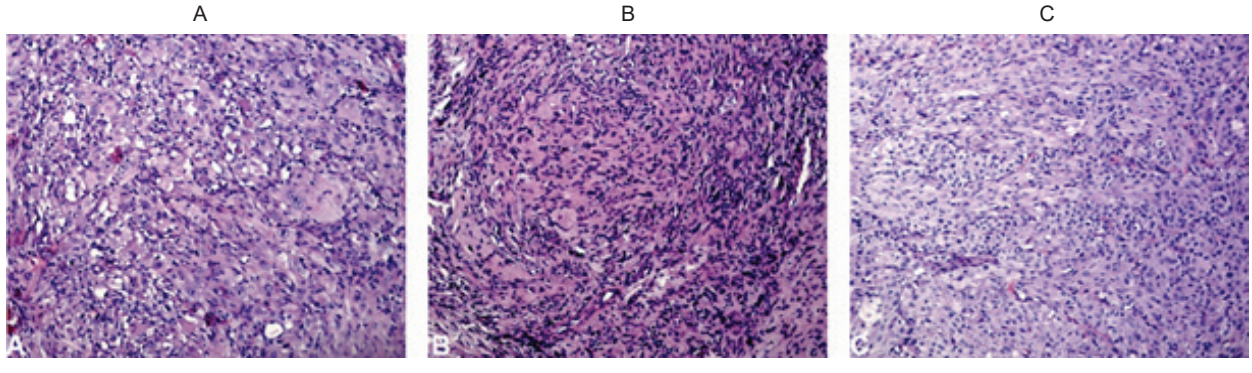

Fig. 4. Histological preparations of subcutaneous xenografts of the U87 glioblastoma cell line. A – 1st group; B – 2nd group; C – 3rd group (control). Magnification ×200

Despite the encouraging results, limitations of the study remain, such as the use of only one glioblastoma cell line and a subcutaneous xenograft model that does not fully recapitulate the brain tumor microenvironment. Transcriptomic and proteomic analyses are needed to clarify the mechanisms of tropolone-temozolomide interaction.

These data provide a basis for further evaluation of tropolones in combination therapy for glioblastoma, opening up opportunities to improve treatment efficacy and reduce side effects.

Conclusion. The findings indicate the promise of tropolone derivatives in conjunction with temozolomide for the treatment of glioblastoma. The efficacy of this combination is likely attributable to a synergistic effect involving the activation of caspase-dependent apoptosis, the suppression of antiapoptotic proteins, and the enhancement of oxidative stress. Moreover, the potential to reduce the dosage of temozolomide enables the mitigation of its toxicity. However, further studies are needed to confirm these results, including investigating the molecular mechanisms of tropolone and TMZ interaction and evaluating efficacy in other glioblastoma models. These data provide a basis for developing new approaches to glioblastoma treatment and improving the efficacy of existing therapies.

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