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# Ophiobolin A – in the Treatment of Glioblastoma Multiforme (GBM)

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**Abstract:** Glioblastoma multiforme (GBM) is malignant brain tumor with very fast progress. Despite the development of diagnostic and therapeutic strategies over the last few years, attempts at treatment and achieving a favorable prognosis remain fruitless. This is believed to be due to the fact that GBM is resistant to apoptosis, which is the main goal of chemotherapeutic actions. Therefore, alternative methods that will prove to be effective are sought. There is great interest in the substance ophiobolin A, which, thanks to appropriate interactions, can lead to the death of glioma tumor cells and ensure complete recovery of patients. This paper will present the problem of currently used therapeutic procedures in the case of diagnosed glioblastoma multiforme (GBM) and the search for new alternative methods that bring great promise. In preparing this review, most publications from recent years were selected, but older publications were not excluded.

**Keywords:** ophiobolin A; glioblastoma multiforme; Paal-Knorr reaction; paraptosis; apoptosis resistance.



## 1. Introduction

Glioblastoma multiforme (GBM) is the primary malignant brain tumor [1] and accounts for 16% of all central nervous system and primary brain tumors [2]. According to the WHO classification, it belongs to the group of grade IV tumors. It occurs more often in men than in women and more often in white people than in other races [3]. There is an increasing trend of GBM in older people. The median for GBM is 64 years [2]. According to the data, the 5-year survival rate for glioblastoma multiforme is 7.2% [4]. Moreover, the development of gliomas is more frequent in patients with genetic syndromes, such as neurofibromatosis type I and II (NF1, NF2), Li-Fraumeni syndrome, or Hippel-Lindau disease [5], which are one of the risk factors for the development of GBM, in addition to ionizing radiation [6]. However, radiation received during imaging studies has not been shown to increase the incidence of this CNS tumor [7-9]. However, radiotherapy used in oncological patients increases the risk of GBM in the future [10]. GBM can occur de novo or through the transformation of a low-grade tumor into glioblastoma multiforme. Primary occurrence is much more common, associated with a

worse prognosis [11]. GBM is located mainly in the brain, but its location has also been observed in the brainstem, spinal cord, and cerebellum. The primary form of glioblastoma multiforme is most often located in the frontal and temporal lobes and least often in the occipital lobe [American Association of Neuroscience Nurses [AANN], 2014; 3]. GBM is characterized by rapid growth and infiltration along blood vessels, nerve fibers, and meninges. It rarely metastasizes outside the central nervous system, but it is possible. Then, it most often metastasizes to the pleura and lungs [12,13].

## 2. Materials and Methods

### *2.1. Clinical image.*

The clinical picture of a patient with a suspected tumor mass in the central nervous system varies depending on the location and size of the tumor [14]. Most often, patients report headaches, nausea, and vomiting resulting from increased intracranial pressure [3]. Paresis, cerebellar symptoms, and neurological disorders, including epileptic seizures, which may affect up to 50% of patients, may occur [15,16].

### *2.2. Diagnosis.*

To diagnose glioblastoma multiforme, it is necessary to confirm changes in imaging studies, i.e., magnetic resonance imaging and computed tomography [3]. In the case of clinical symptoms, a computed tomography (CT) scan is performed [1]. When features indicating the presence of a tumor mass are found, an MRI is performed. The standard in diagnostics and treatment of patients is magnetic resonance imaging (MRI) [1]. After gadolinium administration, a hypointense image of necrosis is observed, along with the visualization of a mass with a dense ring of enhancement [3]. Various MRI sequences (native T1-weighted (T1w), contrast-enhanced (T1CE), T2-weighted (T2w), T2-fluid-attenuated inversion recovery (T2-FLAIR) sequences) indicate the processes occurring in the environment of the developing brain tumor [1]. Usually, at the time of diagnosis, the tumor size is 4 cm [17,18], and the final diagnosis is made based on a histopathological examination of the intraoperatively resected tumor. [4,17].

### *2.3. Treatment methods.*

The treatment of choice for GBM diagnosis is neurosurgery with adjuvant radiotherapy and chemotherapy [National Comprehensive Cancer Network [NCCN], 2015]. Fluorescence is used in surgical treatment by administering 5-aminolevulinic acid [5-ALA] to the patient before surgery [6]. Cancer cells begin to accumulate contrast, and when the surgical field is illuminated with red light, they begin to glow. This procedure, using 5-ALA, is approved in Europe but has not been approved in the United States [19]. In chemotherapy, the first-line drug is TMZ - temozolomide (4-methyl-5-oxo-2,3,4,6,8-pentazabicyclo [4.3.0] nona-2,7,9-trien-9-carboxamide) [6]. It is registered for use by the Food and Drug Administration (FDA) and the Occupational Exposure to Hazardous Drugs (OSHA) [6]. Due to the fact that the tumor is often located in the areas of the brain where the centers of speech, senses, or motor functions are located, complete resection of the tumor is often impossible [3]. The use of standard treatment methods does not meet the expectations of modern medicine and is associated with a poor prognosis. Data show that < 5% of patients with GBM survive 5 years after diagnosis

[20], and about 70% of patients with GBM experience disease progression within a year of diagnosis [21]. The average value of the median survival of patients who received surgical treatment with adjuvant chemotherapy and radiotherapy is only 14.6 months [22]. Newer and newer solutions are emerging in the form of immunotherapy and virotherapy. Numerous tumor-host interactions are sought, and then immunogenic cell death is led by oncolytic viruses [23]. New antibodies, chimeric antigen receptor T cells, and checkpoint inhibitors are used [24]. A monoclonal antibody that has been registered for the treatment of glioblastoma multiforme is pembrolizumab [6]. This humanized monoclonal antibody blocks the binding of the PD-L1 protein (programmed death ligand 1) to the programmed cell death receptor.

*2.4. Resistance to apoptosis.*

Studies show that glioblastoma multiforme relapses may be due in part to the fact that the cancer cells are resistant to apoptosis [25-27]. This is due to genetic changes that affect the PI3K-PTEN-Akt signaling axis and tyrosine kinases [28]. Attention is also paid to effector and regulatory molecules, such as TP53, and their role in the process of programmed cell death [25, 29].

## 3. Results and Discussion

*3.1. New coming – ophiobolin A.*

Great hopes are placed on alternative methods of treating GBM. Such a substance is ophiobolin A (figure 1), a metabolite of the fungus of the Bipolaris species [28]. Numerous sources indicate it can be used as an anticancer agent [30-34]. Due to the phenomenon of cytotoxicity, it causes cell death in nanomolar concentrations [30,35]. This fungus can attack corn, rice, sorghum [35,36], and ophiobolin A, being a phytotoxin of this fungus, blocks the transport of a 6-carbon sugar, which is hexose in these plants [35]. Additionally, it inhibits the activity of the calmodulin protein [35]. It affects the permeability of the cell membrane to potassium ions [36]; however, this relationship with human cancer cells still remains unexplored. Ophiobolin A is active against glioma cells by inducing paraptosis, a type of non-apoptotic cell death [37,38]. Vacuolization, mitochondrial enlargement, and endoplasmic reticulum (ER) enlargement occur [37,38]. There is no DNA strand fragmentation, PARP [poly(ADP-ribose)polymerase] cleavage, and caspase activation [37,38]. However, there is a need for new protein synthesis [38]. A probable mechanism explaining the phenomenon of paraptosis and vacuolization of the above-mentioned structures is the disturbance of potassium ion flow through the calcium-activated potassium channel (BKCa) [39]. In order to maintain the resting potential, sodium and potassium ion flow on both sides of the cell membrane is necessary. The use of ophiobolin A leads to the blocking of BKCa channels, which induces an increase in potassium ions inside the cell [40,41]. In order to maintain homeostasis, water molecules flow into the cell, which leads to swelling and vacuolization of the above-mentioned structures [40,41]. To provide compensation, cells expel vacuoles by exocytosis [40,41]. The subsequent depolarization leads to the influx of calcium ions through L-type calcium channels and an increase in their concentration [40]. The increase in calcium levels also occurs in another mechanism, which involves IP3 and ryanodine receptors [40,41].

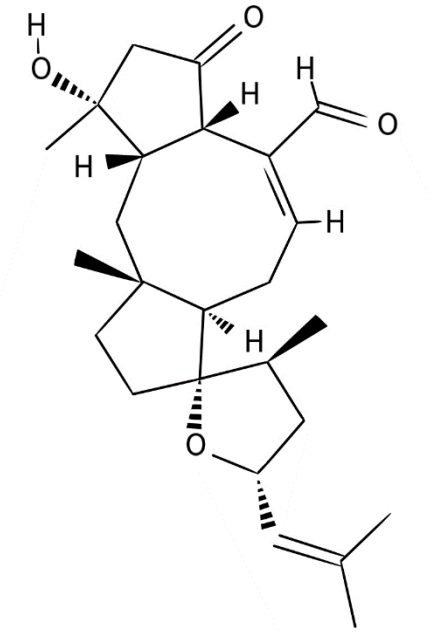

**Figure 1.** Ophiobolin A.

3.1.1. Research on ophiobolin A.

In the conducted study [40], it was shown that ophiobolin A inhibits the proliferation of GBM cells. GBM cell lines U373-MG and T98G and primoculture GL19 were used, which contained markers CD44 and nestin [40]. Additionally, U373-MG and T98G had a mutated p53 gene [40], which made them more resistant to apoptosis [42,43,44]. The MTT colorimetric test was used for these cell lines, and the $IC_{50}$ (inhibitory concentration) was determined [40]. U373-MG, T98G, and GL19 were 0.87 μM, 1.9 μM, and 3.7 μM, respectively [40]. By calculating the global growth rates (GG), it was observed that the cells treated with ophiobolin A achieved lower growth rates compared to the control group cells [30]. Moreover, flow cytometry (FCM) showed that ophiobolin A inhibited the U373-MG cell cycle [40]. After the application of 1 μM OP-A for 72 hours, changes in the morphology of U373-MG cells were observed, which indicates the ongoing changes in the cytoskeleton within the actin filaments [40]. It was noted that after 30 hours of ophiobolin A incubation, there was a significant increase in the number of cells with globular actin but without fibrillar actin [30]. Additionally, the intracellular calcium concentration was determined in U373-MG cells, both in those treated with ophiobolin A and those not exposed to it [40]. It was noted that in U373-MG cells under the influence of OP-A, after 6 h, there was an increase in F-actin and intracellular calcium levels [40]. Moreover, it was described that the administration of 1 μM OP-A reduced the migration of U373-MG cells within 24 h ($p < 0.001$) [40]. In addition to changes in the cytoskeleton, after applying 1 μM OP-A after 6 h, an increase in vacuolation processes was observed in GBM-U373-MG and T98G cell lines and GL19 primoculture [40]. MitoTracker and ER-Tracker dyes were used, and the applied fluorescence showed that vacuolation caused by ophiobolin A involved both mitochondria and the endoplasmic reticulum (ER) [40]. The data showed that the vacuolation that occurred is characteristic of paraptosis [37]. Moreover, after staining with acridine orange (AO), it was noted that the number of cells treated with ophiobolin A that stained red did not increase [40]. These structures were not autophagic [40]. Additionally, the number of cells stained green also did not increase, indicating that ophiobolin A did not increase the permeability of the lysosomal membrane [40]. The annexin V test was used to confirm that ophiobolin A is not associated with apoptotic cell death [40]. It was found

that there was no difference in the percentage of cells that showed the presence of annexin V between the control group and the research group that was treated with OP-A at a dose corresponding to the $IC_{50}$, i.e., the level that causes 50% inhibition of cell growth [30]. Necrotic cell death was also excluded by using PI staining [40]. Using an immunoblot test, it was shown that the death of U373-MG cells, treated with ophiobolin A, does not depend on the participation of caspases and does not cause PARP cleavage - which indicates the mechanism of paraptosis [40]. Moreover, after the use of cycloheximide (CHX) - 0.25 μg/ml in the U373-MG cell line, a reduction in the level of cell death caused by ophiobolin A was noted ($p<0.001$) [40]. This is another argument proving that OP-A causes cell death by paraptosis. The fact that ophiobolin A inhibits proton excretion due to changes in permeability to potassium ions prompted researchers to determine the importance of calcium-activated potassium channels for the paraptosis process. It is known that in glioblastoma multiforme cells, there is a high expression of BKCa channels [40,45]. By means of immunofluorescence pseudoconfocal microscopy using a specific antibody, the localization of BKCa channels was determined in the U373-MG cell line [40]. Their presence was demonstrated in mitochondria, endoplasmic reticulum, cell nucleus, and also along the cell membrane [30]. In order to confirm the functional BKCa channels in GBM cells, a selective inhibitor of calcium-activated potassium channels - iberiotoxin [46] was used. It was shown that ophiobolin A affects the changes in the modulation of BKCa channels by reducing their activity due to the fact that it is their inhibitor in GBM U373-MG cells [40]. It enhances non-apoptotic cell death and reduces cell migration and proliferation [40].

*3.2. In vivo study – pilot study.*

In a pilot in vivo study [46] in mouse models of GBM, 10 mg/kg of ophiobolin A was administered three times a week for seven weeks or 21 days. It was shown that mice with U251-LUC tumors had statistically significant survival and reduced tumor growth [46]. It was also shown that ophiobolin A can cross the blood-brain barrier. A metabolite of ophiobolin A was injected into the tail vein of mice with tumors [46]. After 15 minutes of administration, its presence in the tumor was noted in an unchanged form, which indicates that the barrier between blood vessels and nervous tissue had been crossed [46].

3.2.1. Paal-Knorr reaction.

The Paal-Knorr reaction is a type of synthesis reaction, the mechanism of which is not yet fully understood. It enables the formation of heterocyclic furan, pyrrole, and thiophene rings from 1,4-diketones [22]. Depending on the reagents used - i.e., acid, amine, or sulfur compound - furans, pyrroles, and thiophenes are formed [46]. The studies carried out confirmed that ophiobolin A reacts with primary amines and gives adducts containing pyrrole [46].

3.2.2. In vivo study.

The study [46] noted that ophiobolin A in acidic environments reacts with alcohols and forms bis-acetals. In the following reactions, hydrazine and acetic anhydride form aromatic rings: pyridazine and furan [46]. After using Wittig reagents from the diastereomer at C21 α,β-unsaturated esters and nitrile were obtained [46]. After dehydration, C3- and C4-alkene were obtained. Next, the reactivity of alkene C18 C19 was tested, and ophiobolin A was treated with bromine [46]. As for the biological significance of the Paal-Knorr reaction - its participation in

the axonal loss caused by n-hexane in the CNS is stated [46]. 2,5-Hexanedione, a toxic n-hexane metabolite, undergoes condensation with lysine residues and forms 2,5-dimethylpyrrole in neurofilaments, as shown in vivo and in vitro studies [47]. In the study [46], pyrrole was obtained as a product, and the analysis of the compounds was conducted. The MTT colorimetric assay was used, and 5 cancer cell lines were analyzed, including GBM U373 [48], melanoma SKMEL-28 [49], non-small cell lung cancer (NSCLC) A549 [50], anaplastic oligodendroglioma Hs683 [48], breast cancer MCF-7 [51]. After data analysis, it was shown that the compounds obtained by modification of the C18 C19 alkene had antiproliferative properties [47]. It was also noted that the C5, C21-dicarbonyl structure has antiproliferative effects [47]. The discovery that ophiobolin A reacts with primary amines suggests the pyrolization of lysine residues, which may be necessary for further studies to investigate the significance of the Paal-Knorr synthesis reaction [47].

## 4. Conclusions

The obtained in vivo results give hope for the future use of ophiobolin A in treating glioblastoma multiforme as an anti-GBM substance. It seems that the use of ophiobolin A will make it possible to overcome the problem of resistance of cancer cells to apoptosis in the case of using classical therapeutic methods, thanks to its properties. Currently, the knowledge is insufficient to answer the questions regarding the efficacy, safety, and future widespread use of the metabolite of the fungus Bipolaris. Further studies are necessary to allow for a more precise determination of new properties of ophiobolin A, mechanisms of its action, and cellular targets of this substance.

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## Conflicts of Interest

The authors declare no conflict of interest.

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