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ORIGINAL ARTICLE

Evidence for the efficacy of disulfiram and copper combination in glioblastoma multiforme - A propos of a case

Petros N. Karamanakos[1], Dimitrios T. Trafalis[2], Dionysios J. Papachristou[3], Eleftheria S. Panteli[4], Maria Papavasilopoulou[5], Andreas Karatzas[5], Dimitrios Kardamakis[6], Georgios Nasioulas[7], Marios Marselos[8]

[1]Department of Neurosurgery, Olympion General Hospital and Rehabilitation Center, Patras, Greece; [2]Laboratory of Pharmacology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; [3]Department of Anatomy-Histology-Embryology, Unit of Bone and Soft Tissue Studies, University of Patras Medical School, Patras, Greece; [4]Department of Anesthesiology and Critical Care Medicine, University Hospital of Patras, School of Medicine, University of Patras, Rion-Patras, Greece; [5]Department of Radiology, Olympion General Hospital and Rehabilitation Center, Patras, Greece; [6]Department of Radiation Oncology, University of Patras Medical School, Patras Greece; [7]Department of Molecular Biology, GeneKor Medical S.A., Gerakas, Greece; [8]Department of Pharmacology, Medical School, University of Ioannina, Ioannina, Greece

Summary

Glioblastoma multiforme (GBM) is the most common and aggressive malignancy of the central nervous system. Treatment usually involves a combination of surgical resection, chemotherapy, and radiotherapy, but ultimately this condition is incurable. Besides the dismal prognosis of GBM, financial factors have also presented challenges for advancing treatments. Taking into consideration the high cost of developing new anticancer drugs as well as the fact that GBM is a rare disease, thus further limiting financial incentive for drug development, it becomes obvious that there has been growing interest for repurposing candidates. One of the most promising drugs to repurpose for treating GBM is disulfiram (DSF). DSF is a relatively nontoxic drug used for more than sixty years in the treatment of chronic alcoholism with the ability to readily cross the blood–brain barrier. Repurposing DSF for use as an anticancer drug in general has recently become of interest because of its preclinically described anticancer effects against various human cancers. Interestingly, a number of these effects were shown to be copper (Cu)-dependent. The purpose of this paper was to review the existing literature surrounding preclinical and clinical data on the effects of DSF -alone or in combination with Cu- in GBM. In addition, we present the first case of a GBM patient safely treated with DSF/Cu combination along with standard therapy exhibiting remarkably increased progression-free (PFS) and overall survival (OS).

Key words: *copper, disulfiram, glioblastoma multiforme, temozolomide, survival*

Introduction

GBM, a WHO grade IV glioma, is the most common and most aggressive malignant primary brain tumor in adults and among the most aggressive of all tumors [1]. Despite maximal treatment, consisting of surgical resection followed by concurrent radiation and temozolomide (TMZ) therapy, tumor relapse occurs regularly accompanied by unfavourable prognosis. Likewise, the overall median PFS and OS for GBM patients are approximately 7 and 15 months, respectively [2,3]. The presumed cause of GBM recurrence is a sub-population of tumor stem cells (TSCs) that have been shown to confer resistance to radio- and chemotherapy [4-9], either because of distinct biophysical and genetic properties, or possibly due to migration outside of the treatment field [4,10]. Tumor heterogeneity and diffuse invasiveness comprise two other barriers to successful treatment of GBM.

Correspondence to: Petros N. Karamanakos, MD, PhD. Department of Neurosurgery, Olympion General Hospital and Rehabilitation Center, Volou and Milihou street, 26443, Patras, Greece.
Tel: +30 6945548463, Fax: +30 2610464115, E-mail: pkaramanakos@olympion-sa.gr, me00188@cc.uoi.gr
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Consequently, confronting the infiltrative character of GBM and targeting TSCs as the potential cause of tumor recurrence clearly represents an opportunity to improve GBM patient prognosis.

Besides the dismal prognosis of GBM, financial factors have also presented challenges for advancing treatments. Taking into consideration the high cost of developing new anticancer drugs as well as the fact that GBM is a rare disease [11], thus further limiting financial incentive for drug development, it becomes obvious that there has been growing interest to repurpose older drugs approved for non-oncologic diseases as potential treatments for GBM. One of the most promising drugs to repurpose for treating GBM is DSF. DSF is a relatively nontoxic dithiocarbamate disulfide with well-established pharmacokinetics, and ability to readily cross the blood-brain barrier which has been used for more than sixty years in the treatment of chronic alcoholism, because of the unpleasant symptoms it provokes after ethanol intake. The underlying mechanism is believed to be the accumulation of acetaldehyde in the blood, due to inhibition of the liver aldehyde dehydrogenases (ALDHs). The original suggestion to use DSF in the treatment of GBM came from a position paper published in 2009 [12] associating high ALDH expression in GBM TSCs with the ability of these cells to repopulate a tumor mass after primary therapy. According to the authors of this paper, inhibition of TSCs ALDH by DSF would make them less able to regenerate a stem cell derived tumor mass after resection and radio-chemotherapy. Such a role for DSF was later supported by a number of preclinical *in vitro* and *in vivo* studies [13-18]. Interestingly, a number of these anti-GBM effects of DSF were shown to be copper (Cu)-dependent [13,15,18]. Based on the promising preclinical activity of DSF against GBM a number of clinical trials are currently underway.

The purpose of the present paper was to review the existing literature regarding the preclinical and clinical data on the effects of DSF -alone or in combination with Cu- in GBM. In addition, we report the first case of a 37-year-old male with a left parieto-occipital multifocal GBM who underwent partial resection of the tumor whereafter he received DSF and Cu gluconate along with the standard radio-chemotherapy and achieving increased PFS and OS.

Case presentation

A 37-year-old male was admitted to our Center due to a history of headache, absence of seizures and mild mixed aphasia of almost 3-month duration. The neurological examination on the day of admission revealed only mild expressive aphasia and he was graded as Karnofsky Performance score (KPS) 90. He underwent brain CT and contrast-enhanced MRI, which disclosed two inhomogeneous lesions in the left parieto-occipital area with surrounding edema. The larger lesion measuring 4.7 cm was cystic, while the smaller measuring 2 cm was mixed cystic-solid (Figure 1A).

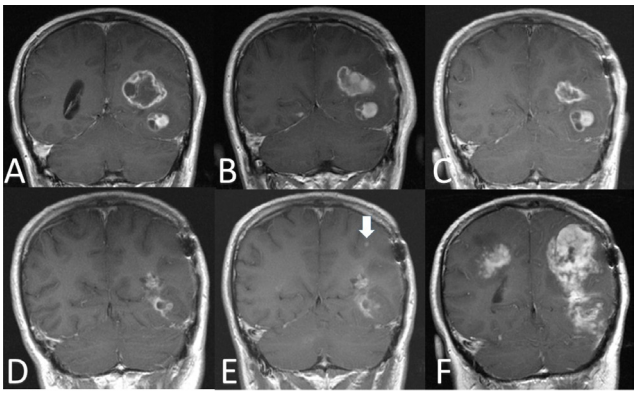

Figure 1. Serial coronal T1 weighted brain MRI images with contrast enhancement. **(A)** preoperative MRI, **(B)** postoperative MRI, **(C)** MRI 3 months after the operation, **(D)** MRI 12 months after the operation, **(E)** MRI 15 months after the operation, **(F)** MRI 20 months after the operation.

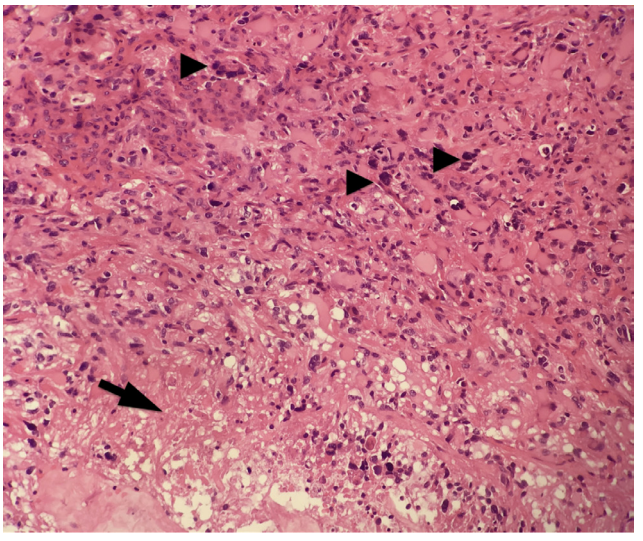

Figure 2. Microphotograph of the glioblastoma multiforme. Note the significant cellular pleomorphism (arrowheads) and the areas of necrosis (arrow), characteristics of high (grade IV) tumor (hematoxylin and eosin, original magnification 20X).

The patient underwent a left occipito-parietal craniotomy with partial resection of the largest lesion (about 50%, Figure 1B). He had an uneventful postoperative recovery and he was discharged from the hospital in good neurological condition on the 12th postoperative day. The histopathological examination showed GBM (Figure 2), while the O-6-methly-guanly-methyl-transferase (MGMT)-promoter methylation as well as isocitrate dehydrogenase (IDH)1-mutational status were also

analyzed and revealed that the patient had an unmethylated MGMT promoter and a mutated IDH1. He received the standard adjuvant radio-chemotherapy including 30 daily fractions of irradiation with a total dose of 60 Gy and concomitant TMZ 75 mg/m^2 whereafter he started maintenance TMZ (200 mg/m^2) on Days 1-5 every 28 days (5/28 cycle). Maintenance TMZ (150-200 mg/m^2) is generally given for 6 5/28 cycles. However, due to good tolerance our patient received 19 cycles. In addition, after the histopathological confirmation of the diagnosis and before the initiation of radio-chemotherapy the patient started DSF (250 mg x 1, p.o) along with Cu gluconate (3 mg x2, p.o). DSF treatment had to be stopped twice for 15 days each time due to transient elevation of transaminase levels.

A brain contrast-enhanced MRI was carried out monthly and revealed an excellent radiological (Figure 1C-1D) and clinical response to radio-chemotherapy the first 12 months after the operation. The patient remained symptomless and without radiological signs of disease progression for 15 months after the operation when the first sign of radiological recurrence appeared but not in the initial tumor location (Figure 1E). He continued to be symptomless for the next 3 months when he presented with headache and mild aphasic disorders and the brain MRI 20 months after the operation revealed two new space-occupying lesions away from the original tumor bulk (Figure 1F). For this reason he underwent 3 sessions of stereotactic radiosurgery with Cyberknife (2700 cGy) which somehow controlled the disease but he died 5 months later (26 months after the operation).

Discussion

In the quest for effective therapies for human cancer, it is occasionally possible to apply an already approved drug toward a new use. This strategy has been most commonly used to apply cancer chemotherapeutic agents approved for one type of malignancy to the treatment of others but may also lend itself to antineoplastic application of older drugs approved for non-oncologic diseases. In this context DSF seems to be a promising drug to repurpose for treating GBM.

GBM, like many other cancers, show a subpopulation of ALDH overexpressing TSCs [20-25]. More specifically, and as it was recently shown, ALDH1A1, a cytoplasmatic isoform of ALDH, is a novel stem cell marker in human GBM [26]. As a member of the ALDH enzyme family, ALDH1A1 catalyzes the oxidation of intracellular aldehydes including the transformation of retinol to retinoic acid (RA). RA is a modulator of cell proliferation and differentiation that possibly contributes to the maintenance of an undifferentiated stem cell phenotype. Indeed, Rasper et al. [26] showed that high protein levels of ALDH1A1 facilitated neurosphere formation in established GBM cell lines keeping tumor cells in an undifferentiated, stem cell-like state. On the other hand, they showed that inhibition of ALDH1A1 *in vitro* decreased both the number of neurospheres and their size, inducing premature cellular differentiation and reducing clonogenic capacity. In addition, ALDH1A1 has been shown to be a mediator for resistance of GBM to TMZ and a reliable predictor of clinical outcome; prognosis of patients with a high level of ALDH1A1 expression was poor compared with that of patients with low levels [27]. Consequently, ALDH1A1 may serve as a potential target to improve treatment of human GBM through inhibition of the enzyme.

During the course of a research project in our laboratory, we investigated all known ALDH inhibitors in Wistar rats and we concluded that among these inhibitors DSF possesses the strongest inhibitory effect on ALDH1A1 [28]. In a position paper and based on our experimental work, Kast and Belda-Iniesta [12] hypothesized that DSF, as an inhibitor of TSCs ALDH, might be of importance in the treatment of GBM. According to their hypothesis and since ALDH function is used by cancer stem cells to repopulate a tumor mass after radio-chemotherapy cytoreduction, inhibition of TSCs ALDH will make them less able to regenerate a stem cell derived tumor mass after primary resection and radio-chemotherapy. This was actually the first report in the literature regarding a possible implication of DSF in the treatment of GBM.

In another paper published in 2012, Liu et al. [13] showed that the combination of DSF and copper (Cu) was cytotoxic on human GBM cell lines and ALDH-positive TSCs in a Cu-dependent manner. The cytotoxic effect of DSF/Cu combination was attributed to the induction of reactive oxygen species (ROS) and inhibition of both ALDH and Nuclear Factor kappa B (NFkB) pathway.

In addition, Triscott et al. [14] concluded that DSF completely suppressed GBM cell growth *in vitro* and the same degree of inhibition (~100%) was observed in self-renewal assays. Of notable importance, they showed that DSF was highly effective in situations where cells had developed TMZ resistance. Moreover, it was shown that DSF suppressed the growth and self-renewal of primary cells from two GBM tumors; these cells were resistant to TMZ and had unmethylated MGMT. Interestingly, these actions of DSF were attributed

to inhibition of the enzyme polo-like kinase 1 (PLK1) rather than to inhibition of ALDH. PLK1 is a key serine/threonine kinase that has been demonstrated to be a promising therapeutic target for brain tumors as it is highly overexpressed in cancer compared to normal tissue cells [29].

In another experimental work published in 2012, Hothi et al. [15] screened a diverse chemical library of 2000 compounds that repeatedly inhibited cellular proliferation and identified DSF as a potent inhibitor of multiple patient-derived GBM TSCs proliferation. They demonstrated that the antitumor effects of DSF were a result of proteasome inhibition and the subsequent induction of tumor cell death, while a role of ALDH inhibition was also suggested. In addition, they showed that DSF activity was dependent on the presence of Cu ions, consistent with the formation of a thiocarbamate-Cu complex that functions as a proteasome inhibitor. According to the authors, these findings combined with the fact that proteasome inhibitors are emerging as promising therapeutic agents against glioma [30] render the proteasome inhibitor DSF an attractive agent to test in clinical trials for GBM patients.

MGMT is a DNA repair protein and chemotherapy target that removes the mutagenic O-6-alkyl groups from guanines, and thus confers resistance to alkylating agents (such as TMZ) in brain tumors; epigenetic silencing of the MGMT DNA-repair gene by promoter methylation compromises DNA repair and has been associated with longer survival in GBM patients who receive TMZ therapy [31]. In 2014, Paranjpe et al. [16] showed that DSF is a direct and potent inhibitor of MGMT in human brain tumor cells and normal mouse brain, while a preferential inhibition of tumor MGMT was observed in nude mice bearing human T98 GBM xenografts.

In a separate study published in 2013, Westhoff et al. [17] demonstrated that the invasive phenotype of GBM is orchestrated by the transcription factor NF-κB which, via metalloproteinases (MMPs), regulates Fibronectin (Fn) processing. Both, cell lines and TSCs from primary GBM, secrete high levels of Fn and when cleaved by MMPs form an extracellular substrate. Subsequently, forming and interacting with their own microenvironment, GBM cells are licensed to invade their surroundings. NF-κB inhibition, either genetically or pharmacologically by treatment with DSF, significantly abolished the invasive phenotype in the chick chorioallantoic membrane assay. Furthermore, having delineated the underlying molecular mechanism of GBM invasion, the potential of a DSF-based therapy was revealed in a highly invasive orthotrophic GBM mouse model where it was shown that tumor bulk, as well as range and amount of micrometastasis were clearly reduced by DSF treatment.

Finally, in a recent paper by Lun et al. [18], it was shown that low doses of DSF and Cu significantly augmented TMZ activity *in vitro*, and importantly, prolonged *in vivo* survival of mice with orthotopic GBM tumors. Moreover, it was found that in addition to acting as a potent proteasome inhibitor, DSF-Cu functionally impairs DNA repair pathways and enhances the effects of DNA alkylating agents and radiation. These observations suggest that DSF-Cu inhibits proteasome activity and augments the therapeutic effects of DNA-damaging agents (TMZ and radiation).

Based on the favorable preclinical data of DSF use against GBM there are three ongoing clinical trials where DSF and Cu along with standard therapy are tested in newly diagnosed GBM (www.clinicaltrials.gov, identifiers NCT01777919 and NCT02715609) and recurrent GBM patients (www.clinicaltrials.gov, identifier NCT02678975). Furthermore, the results of a phase I clinical trial of daily DSF use in combination with standard therapy but in the absence of concurrent Cu administration in newly diagnosed GBM patients have been already published [19]. In this 12-patient study, the maximum tolerated dose of DSF was determined to be 500 mg per day with a median PFS of 8.1 months, while data on OS were not available.

In the present manuscript we presented the first case of a patient with newly diagnosed multifocal GBM who underwent partial resection of the tumor and received DSF and Cu gluconate along with standard radio-chemotherapy lacking major toxicity and resulting in increased PFS and OS. More specifically, PFS and OS in our case were 15 and 26 months respectively when the reported median OS for GBM patients with similar characteristics (multifocal tumors/partial resection) is 5 months [32]. We do not disregard the fact that our patient was young and with good initial KPS which are widely recognized as favorable prognostic factors for longer survival [33]. In addition, the presented case had a mutated IDH1 which has been also associated with improved survival in GBM patients [34], even though its independent prognostic impact is still unknown and recently questioned [35]. Furthermore, our patient underwent stereotactic radiosurgery which might have partly contributed to the prolonged survival. On the other hand, he had an unmethylated MGMT promoter which has been correlated with increased resistance to TMZ and worse outcome in GBM patients

[31,36], while the DSF dosage of 250 mg/day used is by far below the maximal recommended daily dose of 500 mg.

Interestingly, and despite the fact that the majority of GBMs (>90%) recur at the site of the initial tumor [37,38], in our case the recurrence took place away from the original tumor bulk. Whether this observation implies a role for whole brain radiation instead of local radiation to prevent tumor recurrence in GBM patients treated with the suggested pharmacological scheme remains to be evaluated in the context of a clinical trial.

In conclusion, there is ample preclinical evidence for the efficacy of DSF-Cu combination against GBM. In addition, we presented the first case of a GBM patient safely treated with DSF and Cu gluconate along with standard therapy exhibiting remarkably increased survival. Whether the anti-GBM mechanism of action of DSF and Cu is based on ALDH inhibition, MGMT inhibition, NFkB inhibition, proteasome inhibition, increased intracellular ROS generation, or on a combination of actions is currently unknown. In any case, and due to the fact that GBM is a disease of heterogeneity with devastating outcomes, chemotherapy with multi-targeting properties and financial advantages such as DSF may be the way of the future. Moreover, DSF has been used safely in humans for over half a century and therefore we believe it has excellent potential to be repurposed for the treatment of GBM. However, further preclinical and clinical studies are warranted addressing the efficacy and mechanism of action of DSF/Cu combination in GBM.

Conflict of interests

The authors declare no confict of interests.

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