Methadone-mediated sensitization of glioblastoma cells is drug and cell line dependent

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Abstract

Purpose d,l-methadone (MET), an analgesic drug used for pain treatment and opiate addiction, has achieved attention from oncologists and social media as possible chemoensitizing agent in cancer therapy, notably brain cancer (glioblastoma multiforme, GBM). MET has been reported to enhance doxorubicin-induced cytotoxicity in GBM cells via activation of the µ-opioid receptor (MOR). Here, we extended this work and quantified the toxic effect of MET in comparison to other opioids alone and in combination with doxorubicin and the clinically more relevant alkylating drug temozolomide (TMZ), using a set of GBM cell lines and primary GBM cells.

Methods MOR expression in GBM cells was investigated by immunofluorescence and immunoblotting. Resistance to drugs alone and in combination with anticancer drugs was assessed by MTT assays. Concentration effect curves were fitted by nonlinear regression analysis and IC₅₀ values were calculated. Apoptosis and necrosis rates were determined by annexin V/propidium iodide (PI)-flow cytometry.

Results MET alone was cytotoxic in all GBM cell lines and primary GBM cells at high micromolar concentrations (IC₅₀ ~ 60–130 µM), observed both in the metabolic MTT assay and by quantifying apoptosis and necrosis, while morphine and oxycodone were not cytotoxic in this concentration range. Naloxone was not able to block MET-induced cytotoxicity, indicating that cell death-inducing effects of MET are not MOR-dependent. We recorded doxorubicin and TMZ concentration-response curves in combination with fixed MET concentrations. MET enhanced doxorubicin-induced cytotoxicity in only one cell line, and in primary cells it was observed only in a particular MET concentration range. In all assays, MET was not effective in sensitizing cells to TMZ. In two cell lines, MET even decreased the cell’s sensitivity to TMZ.

Conclusion MET was found to be cytotoxic in GBM cells in vitro only at high, clinically not relevant concentrations, where it was effective in inducing apoptosis and necrosis. Sensitizing effects were only observed in combination with doxorubicin, but not with TMZ, and are dependent on cell line and the applied drug concentration. Therefore, our findings do not support the use of MET in the treatment of GBM in combination with TMZ, as no sensitizing effect of MET was observed.

Keywords Glioblastoma · Methadone · Temozolomide · Doxorubicin · Apoptosis · Chemosensitizer

Abbreviations

DOXO Doxorubicin
GBM Glioblastoma multiforme

Gi Inhibitory G-protein
MET d,l-methadone
MOR µ-Opioid receptor
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NAL Naloxone

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Background

Grade IV glioma (glioblastoma multiforme, GBM) is the most aggressive form of brain cancer with the highest incidence among adults (Louis et al. 2016; Siegel et al. 2020). Median survival is 14.6 months under therapy, the 5-year survival rate is only less than 6% (Ostrom et al. 2016), indicating that there is high need for new therapeutic options. Therapeutic standard of care in GBM treatment is radiotherapy with concomitant temozolomide (TMZ) treatment (Stupp et al. 2005, 2009). Previous studies indicate that d,l-methadone (MET), an analgesic drug used for pain treatment and opiate addiction (Krantz and Mehler 2004; Parsons et al. 2010), increases apoptosis of leukemia cells and the cytotoxic effects of the topoisomerase II-inhibitor doxorubicin (Friesen et al. 2008, 2013; Singh et al. 2011). In a follow-up study, the same group showed that the opioid has also the potential to enhance apoptosis induced by doxorubicin in GBM cells (Friesen et al. 2014). The proposed mechanism of action involves activation of the µ-opioid receptor (MOR) and subsequent suppression of cAMP/protein kinase A (PKA) signaling via inhibitory G-proteins (Gi), which finally activates caspases and induces apoptosis. However, cAMP displays pro- and anti-apoptotic effects depending on cell type (Insel et al. 2012), raising the question if all GBM cells respond equally to MET treatment. The proposed mechanism of action involves activation of the µ-opioid receptor (MOR) and subsequent suppression of cAMP/protein kinase A (PKA) signaling via inhibitory G-proteins (Gi), which finally activates caspases and induces apoptosis. However, cAMP displays pro- and anti-apoptotic effects depending on cell type (Insel et al. 2012), raising the question if all GBM cells respond equally to MET treatment. In addition, it was shown that MET increases intracellular doxorubicin levels probably by inhibiting P-glycoproteins (P-gp) in GBM cells (Friesen et al. 2014). On the other hand, data published by others indicate no sensitizing effect of MET on various doxorubicin-treated canine tumor cells (Cueni et al. 2020).

It is important to note that the first-line therapy in GBM treatment is TMZ in combination with radiotherapy, while doxorubicin in first place has no indication for GBM because of its poor blood–brain barrier penetration and neurologic side effects (Merker et al. 1978; Neuwelt et al. 1981). A liposomal formulation of doxorubicin (Caelyx®) is sometimes used off-label with marginal benefit (Fabel et al. 2001; Fiorillo et al. 2004). Despite the lack of preclinical and clinical data on any beneficial effects of MET on TMZ therapy, it has been promoted as promising therapeutic option for GBM treatment.

Considering the importance of TMZ in GBM therapy, we aimed at investigating, first, the cytotoxic effect of MET in a variety of GBM cell lines differing in their p53 status and primary GBM cells and, secondly, to assess the contribution of MET to doxorubicin and TMZ-induced cytotoxicity in these cells. We recorded concentration–response curves in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays of MET alone and doxorubicin or TMZ in combination with MET to quantify effects and determine IC50 values. Furthermore, we compared MET with morphine and oxycodone, other opioids used in oncology, and assessed the effect of the MOR inhibitor naloxone to determine if the opioid-induced cytotoxicity is mediated via MOR. Although MET was cytotoxic at high, clinically not relevant concentrations in all GBM cells, we only observed a weak MET-induced sensitization to doxorubicin in one established cell line and in primary cells, while no effect of MET was observed on TMZ-induced cell death.

Methods

Materials

All compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA). Temozolomide (Temodal®) was dissolved in dimethyl sulfoxide (DMSO), doxorubicin hydrochloride, d,l-methadone hydrochloride, morphine sulfate salt pentahydrate, oxycodone, naloxone hydrochloride dehydrate were dissolved in sterile water.

Cell culture

U87-MG, U251-MG, and U373-MG (Uppsala) GBM cell lines were obtained by Sigma-Aldrich (St. Louis, MO, USA) (HPA Culture Collections). The A172 GBM cell line was obtained by American Type Culture Collection (ATCC, Manassas, VA, USA). Cell lines were not used beyond passage 20. U251 Cells were cultivated in Roswell Park Memorial Institute (RPMI) 1640 medium (Biochrom, Berlin, Germany) containing 10% fetal calf serum (FCS; Biochrom, Berlin, Germany), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Biowest, Nuaillé, France). All other cells and cell lines were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) low glucose (Biowest, Nuaillé, France) containing 10% FCS (Biochrom, Berlin, Germany), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Biowest, Nuaillé, France). All other cells and cell lines were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) low glucose (Biowest, Nuaillé, France) containing 10% FCS (Biochrom, Berlin, Germany), 100 IU/ml penicillin, and 100 µg/ml streptomycin (Biowest, Nuaillé, France). Primary GBM cells derived from a primary GBM tumor biopsy were obtained from the University Hospital Cologne, genetically characterized and cultured as previously described (Haas et al. 2018).

Western blot analysis

In order to prepare whole cell lysates, cells were washed with ice-cold phosphate-buffered saline (PBS; Biowest, Nuaillé, France) and lysed with ice-cold Denaturing Cell
Extraction Buffer (FNN00091, Thermo Fisher Scientific, Waltham, MA, USA), incubated on ice for 30 min, and centrifuged for 15 min at 4 °C. The supernatant was used for protein content determination and subsequent immunoblotting. For immunoblotting standard procedures using the following antibodies were used as previously described (Haas et al. 2009). Anti-MOR-1 (D-12; 1:1000, Santa Cruz Biotechnology, TX, USA) combined with goat antimouse IgG-HRP (1:5000, Santa Cruz Biotechnology, TX, USA) and β-Actin-HRP (C-4; Santa Cruz Biotechnology, TX, USA). Immunoblots were developed with the enhanced chemoluminescence system (Amersham Biosciences, Little Chalfont, United Kingdom).

**MTT assay**

For MTT assays, we followed a published protocol (Eckstein et al. 2009). Briefly, 5000 (A172, U251, U87, U373) or 15,000 (primary) cells were plated on 96 wells and grown at 37 °C and 5% CO2 overnight. Cell survival after exposure to either opioids alone or doxorubicin/TMZ in the presence of MET as indicated was determined by MTT assays after 72 h. In combination experiments MET or naloxone were added to culture medium 1 h prior to addition of compounds for 72 h. Controls were treated with vehicle (DMSO or water). Final DMSO concentrations in media did not exceed 1%.

**Annexin V/propidium iodide (PI) apoptosis assay**

For apoptosis measurements the BD Pharmingen™ FITC Annexin V Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) was used according to the manufacturer’s protocol. Briefly, 2.5 × 10⁵ cells were seeded into 6-well plates and incubated at 37 °C and 5% CO2 overnight. After compound treatment for 72 h, cells were trypsinized and centrifuged for 4 min at 1500 × g. Supernatant was removed, and cells were resuspended in 500 μL binding buffer. 5 μL PI and 5 μL Annexin V-FITC were mixed with 100 μL of cells in binding buffer. After 15 min of incubation on ice, samples were analyzed by flow cytometry (FACScan, Calibur™, BD Bioscience, Franklin Lakes, NJ, USA). Data analysis and statistical methods

Concentration effect curves were fitted to data points by nonlinear regression analysis using the four-parameter logistic equation (GraphPad™ Prism). Top of each curve was defined as 100% and bottom as 0%. Statistical differences between two groups were determined by paired 2-tailed Student’s t test. Comparisons among several groups were performed by ANOVA followed by Tukey post-hoc test. The data are presented as mean ± SEM or ± SD as indicated.

**Results**

It has previously been shown that MOR is expressed in human GBM cell lines and primary cells (Brawanski et al. 2018; Friesen et al. 2014; Oppermann et al. 2019; Vatter et al. 2020). Here, we demonstrate the expression of MOR in commonly used GBM cell lines (U87, U251, U373) and primary GBM cells isolated from a patient biopsy by Western blotting (Fig. 1a) and immunocytochemistry (Fig. 1b), confirming and extending the finding that MOR is expressed in a wide range of GBM cells to a similar extent.

We next asked whether MET is cytotoxic in these MOR-expressing cells, including the A172 cell line in which beneficial effects of MET on doxorubicin-induced cytotoxicity were reported (Friesen et al. 2014). In MTT viability assays, we obtained very similar IC₅₀ values for MET in all GBM cell lines, which were in the range of 62–130 μM, measured 72 h after the onset of treatment. MET displayed a steep concentration response in all cells (Hill slopes ranging from −3.3 to −4.4). The lowest IC₅₀ of 62 μM was obtained for A172 cells (Fig. 2a). Despite differences in p53 status (Table S1), all cell lines were uniformly responding to MET. Annexin V/propidium iodide (AV/PI) flow cytometry analysis of cell lines treated with the previously determined IC₅₀ MET concentrations revealed that approximately 50% of cell deaths each account for apoptosis and necrosis (Fig. 2b).

In order to test if MET-induced cytotoxicity is mediated via MOR, we co-treated A172, U373 and primary cells with the MOR antagonist naloxone and MET. Naloxone treatment alone did not affect cell viability and, most importantly, it was not capable of abolishing MET-induced cytotoxicity...
MET treatment (Fig. 2c–e). The opposite was true, naloxone even significantly increased MET toxicity in A172 and primary cells (Fig. 2f), indicating that MET does not require MOR for its cytotoxic action.

To further verify these findings, we treated cells with other MOR agonists used in clinical practice for pain management such as morphine and oxycodone. Strikingly, both compounds showed very weak cytotoxicity in supra-therapeutic concentrations in all GBM cell lines and primary cells (Fig. 3a–e). The determined IC50 values were in the millimolar range or could not be established because of very low cytotoxicity (Fig. 3f).

Previously, it was reported that various MET concentrations (e.g. 10, 3, 1 µg/mL corresponding to ~30, 10, 3 µM, respectively) were capable of sensitizing A172 cells to a fixed doxorubicin concentration (0.3 µg/mL = 0.5 µM) in apoptosis assays (Friesen et al. 2014). In order to determine a concentration relationship, we treated A172, U87, and primary cells with ascending doxorubicin concentrations combined with 10 µM MET, a concentration which was not cytotoxic, but close to what might be reached in plasma of patients. We chose U87 cells because they do not express P-gp (Haas et al. 2018), while A172 cells likely express P-gp as its inhibition by MET was reported to be responsible for doxorubicin accumulation (Friesen et al. 2014). MTT concentration–response curves overlapped in both tested GBM cell lines and, interestingly also in primary GBM cells (Fig. 4a–c); respective IC50 values showing no significant differences are displayed in Fig. 4d. Of note, in A172 cells at 0.3 µM doxorubicin, combination treatment with 10 µM MET slightly reduced cell viability as compared to doxorubicin treated cells alone (red circle in Fig. 4a). This is close to the concentrations tested in a previous work (0.5 µM doxorubicin combined with 3–30 µM MET), where effects on apoptosis were observed (Friesen et al. 2014).

Next, we repeated this experiment with previously determined IC50 concentrations of MET (Fig. 2a). High dose MET treatment (60 µM) resulted in a slight left shift of the doxorubicin concentration–response curve in A172 cells (Fig. 5a), while curves overlapped in U87 cells at 100 µM MET (Fig. 5b). Also in primary GBM cells at 100 µM MET curves largely overlapped independently of treatment (Fig. 5c). Statistical analysis revealed that MET treatment significantly reduced doxorubicin IC50 values in A172 and primary GBM cells indicating a sensitization (Fig. 5d).

Looking closer at the curves of primary GBM cells, the data revealed at both curves overlap at most data points except at 1 µM (~0.5 µg/mL) doxorubicin (red circle in Fig. 5c), which explains the difference in IC50 values and indicates that a potential synergism of MET and doxorubicin highly depends on cell line and applied drug concentrations.

Doxorubicin is not used in the first-line treatment for GBM. Therefore, we studied the effect of MET on the toxicity of TMZ, which is the clinically relevant drug used in GBM therapy. We treated four GBM cell lines and primary GBM cells with ascending TMZ concentrations in combination with 10 µM MET and performed MTT assays. In A172 cells MET even reduced the sensitivity to TMZ (Fig. 6a) while in all other cells no effect was observed (Fig. 6b–e). The only statistical difference in IC50 values was obtained for A172 cells (Fig. 6f) confirming the negative impact of MET on TMZ treatment in this cell line. Similar results were obtained when cells were treated with an IC50 MET concentration in combination with TMZ (Fig. 7). At this high MET concentrations, both A172 and U373 (Fig. 7a, d, respectively) cells responded in a less sensitive way to TMZ.

Discussion

MET is a widely used therapeutic opioid in narcotic addiction and neuropathic pain syndromes. In oncological settings, it is regularly used as a long-lasting analgesic. Recently, it has also been proposed as a chemosensitizing agent in leukemia and GBM therapy based on the results of in vitro studies and a xenograft mouse model (Friesen et al. 2014, 2008, 2013). In these studies, fixed doxorubicin concentrations combined with MET were applied and MET was found to be capable of sensitizing cells to doxorubicin in terms of apoptosis induction. The proposed mechanism of action rests on activation of MOR, inhibition of P-gp by MET leading to increased intracellular doxorubicin levels, and subsequent induction of apoptosis (Friesen et al. 2014). In another study, the data were not reproduced in doxorubicin and MET-treated canine tumor cells (Cueni et al. 2020), indicating a need for further in vitro studies. MET has also been implicated in enhancing cytotoxic effects of other chemotherapeutics in cancer cell lines of different origins, such as bladder cancer, squamous cell carcinoma and head and neck cancer, albeit with varying efficacy, greatly depending on cell type, the chemotherapeutic agent and applied concentrations (Landgraf et al. 2019; Michalska et al. 2018; Shi et al. 2019). Also for GBM, conflicting results were reported. Thus, a sensitization to acid-based photodynamic therapy (ALA-PDT) by MET was observed in the GBM cell line A172 (Shi et al. 2019, 2020), indicating that MET might also be effective in GBM in combination with other treatments apart from doxorubicin. When MET...
was combined with the more relevant treatment TMZ and irradiation (up to 8 Gy), no beneficial effect was observed in vitro (Oppermann et al. 2019; Vatter et al. 2020), while another study reported agonistic and antagonistic effects at high MET concentrations (> 15 µg/mL) on TMZ, which was cell line-dependent (Brawanski et al. 2018). Interestingly, in
As conflicting results have been reported on the capability of MET to sensitize tumor cells to doxorubicin, we tested the combination of doxorubicin and MET in our cellular system. We used an approach commonly applied to determine sensitizing effects of a compound on cytostatic drugs. We treated cells with a wide range of doxorubicin combined with low (10 µM) and high concentrations (determined IC_{50} concentration for each cell line) of MET for 72 h because at this time point MET already induced cell death. We found a sensitizing effect only for the A172 cell line and to some extent in primary GBM cells, but not for the U87 cell line. We have previously shown that P-gp is not expressed in U87 cells (Haas et al. 2018), which might explain that these cells cannot be sensitized to doxorubicin by MET. Whether inhibition of doxorubicin efflux, as reported for the A172 cell line (Friesen et al. 2014), or inhibition of voltage-gated potassium channels is the underlying reason of MET-induced apoptosis/necrosis remains an open question.

Another important finding of our study is that the sensitizing effect of MET in A172 and primary cells was highly dependent on the applied drug concentrations. Although a complete left shift of the doxorubicin concentration–response curve upon MET treatment only was observed at high, clinically not relevant MET concentrations (60 µM) in A172 cells, lower MET concentrations (10 µM) only further increased doxorubicin cytotoxicity at one single doxorubicin/MET concentration combination but not over the whole concentration range. This might have clinical implications as it is challenging to exactly achieve the needed doxorubicin and MET levels in vivo where synergistic cell death-inducing effects might occur. In addition, tolerable MET plasma levels in addicts are between 0.3 and 1.3 µg/ml (corresponding to ~1–4 µM) after a dose of 60–120 mg/day (Dole and Kreek 1973; Inturrisi et al. 1987) which is much lower as compared to effective in vitro MET concentrations reported previously (Friesen et al. 2014) and in our present study. When a blood–brain barrier penetration of only 42% for MET is assumed (Oldendorf et al. 1972), sufficient brain levels can hardly be reached in GBM patients at feasible MET doses. It could be argued that MET accumulates in tissue stores and also in brain and corresponding tumors (Linares et al. 2015). However, if sufficient MET levels are actually reached in brain tumor tissue at clinically achievable doses warrants further investigation, apart from the fact that doxorubicin is not the indicated treatment option for GBM due to its low blood–brain barrier penetration and neurological side effects (Merker et al. 1978; Neuwelt et al. 1981). Although pegylated and liposomal-encapsulated formulations of doxorubicin (Caelyx®) with increased brain uptake and less side effects are available, GBM is rarely treated with doxorubicin and its liposomal formulations in clinical practice (Fabel et al. 2001; Fiorillo et al. 2004).

Because of these shortcomings, we also tested the clinically more relevant drug TMZ in combination with MET. We did not observe an effect of MET on TMZ-induced...
Fig. 3 Comparison of opioid-induced cytotoxicity in GBM cell lines and primary cells. MTT assays of A172 (a), U87 (b), U251 (c), U373 (d), GBM cell lines and primary cells (e) treated with increasing concentrations of MET, morphine (MOR), and oxycodone (OXY) for 72 h as indicated. Values are displayed as mean ± SD (n = 3–4). f IC\textsubscript{50} values of MET, MOR, and OXY in GBM cells derived from MTT assays, NC not calculated, – not determined.
cytotoxicity in none of the tested GBM cell lines and primary cells, which is in line with a previous study (Oppermann et al. 2019). Importantly, in U373 and A172 cells MET even reduced sensitivity to TMZ, an effect that has also been demonstrated by others in different GBM cell lines (Brawanski et al. 2018). This might be due to cell cycle inhibition following MET treatment especially at high cytotoxic concentrations, which counteracts TMZ-induced cell death responses (Roos et al. 2004). In contrast to other studies where MET and TMZ were applied for 6 days (Brawanski et al. 2018; Kaina et al. 2020; Oppermann et al. 2019), we applied a 72-h MTT protocol as at this time MET already displayed high cytotoxicity and, therefore, we considered it sufficient to observe an effect on TMZ. As a consequence, the determined TMZ IC$_{50}$ values in our study are in the higher micromolar range, as TMZ requires repeated cell cycles to process lesions and activate cell death pathways (He and Kaina 2019). Despite these differences in treatment protocols we yielded similar results, which strengthen the conclusion also drawn by other groups that MET is not capable to synergistically sensitize GBM cell to TMZ. Similar data were obtained on glioblastoma cells and TMZ-induced

Fig. 4 MET (10 µM) does not sensitize GBM cell lines and primary cells to doxorubicin (DOXO). MTT assays of A172 (a), U87 (b) GBM cell lines and primary cells (c) treated with increasing concentrations of DOXO for 72 h pretreated with vehicle or 10 µM MET for 1 h. d IC$_{50}$ values derived from MTT assays of doxorubicin treated GBM cells with or without 10 µM MET. Values are displayed as mean ± SD (n = 3). Note: in A172 at one data point of combination treatment differed from DOXO treatment [0.3 µM, red circle in (a)].
apoptosis and cellular senescence, demonstrating that MET does not impact pathways involved in these endpoints (Kaina et al. 2020).

Conclusions

We conclude that the cytotoxic effect of MET alone on GBM cells is not mediated via the opioid receptor MOR. This implicates that other cellular targets, including voltage-gated potassium channels, are involved, which warrants further investigation. Furthermore, our findings do not support the use of MET in the treatment of GBM in combination with TMZ, as no sensitizing effect of MET was observed. In GBM therapy, treatment with doxorubicin is not the rule. Although we observed in two cell lines (out of 3) a supportive effect of MET on doxorubicin-induced cytotoxicity, we doubt that critical MET concentration levels might be reached in the brain of patients to achieve a potential

![Fig. 5 Sensitization of GBM cells by high MET concentrations to DOXO is cell line-dependent. MTT assays of A172 (a), U87 (b) GBM cell lines, and primary cells (c), treated with increasing concentrations of DOXO for 72 h pretreated with vehicle or the corresponding IC_{50} concentration of MET for 1 h. Values are displayed as mean±SD (n=3) (d) IC_{50} values derived from MTT assays of DOXO-treated GBM cells with or without an IC_{50} concentration of MET; *p<0.05. Note: Combination treatment in primary GBM cells was only statistically different from DOXO alone treated cells due to one data point (red circle in c).](image-url)
Fig. 6 MET (10 µM) does not sensitize GBM cell lines and primary cells to TMZ. MTT assays of A172 (a), U87 (b), U251 (c), U373 (d) GBM cell lines and primary cells (e) treated with increasing concentrations of TMZ for 72 h pretreated with vehicle or 10 µM MET for 1 h. Values are displayed as mean ± SD (n=3–4). (f) IC_{50} values of TMZ (vehicle) and TMZ+MET-treated GBM cells derived from MTT assays; *p < 0.05
Fig. 7 High MET concentrations do not sensitize GBM cell lines and primary cells to TMZ. MTT assays of A172 (a), U87 (b), U251 (c), U373 (d) GBM cell lines and primary cells (e) treated with increasing concentrations of TMZ pretreated with vehicle or the corresponding IC$_{50}$ concentration of MET for 1 h. Values are displayed as mean ± SD (n = 3–4). b IC$_{50}$ values of TMZ (vehicle) and TMZ + MET-treated GBM cells derived from MTT assays; *p < 0.05
synergistic effect with doxorubicin. It is obvious that further clinical studies are warranted.

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Author contributions BH, NE designed and performed experiments. BH, NE, BK analyzed data and wrote the manuscript. JC, SJ, SW performed experiments and analyzed data. All authors read and approved the final manuscript.

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Availability of data and material The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicting/competing interests.

Ethics approval and consent to participate Human tissue specimens of glioblastoma were provided by the tumor tissue bank of the Clinic of Neurosurgery, University of Cologne. The collection of samples was approved by the University’s Institutional Ethical Board. Informed consent of the patients was obtained according to the Helsinki declaration of ethical requirements.

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References

Brawanski K et al (2018) Efficacy of d, l-methadone in the treatment of glioblastoma in vitro. CNS Oncol 7:CNS18. https://doi.org/10.2217/cns-2018-0006

Cueni C, Nytko KJ, Thumser-Henner P, Weyland MS, Rohrer Bley C (2020) Methadone does not potentiate the effect of doxorubicin in canine tumour cell lines. Vet Med Sci. https://doi.org/10.1002/vms3.266

Dole VP, Kreek MJ (1973) Methadone plasma level: sustained by a reservoir of drug in tissue. Proc Natl Acad Sci USA 70:10. https://doi.org/10.1073/pnas.70.1.10

Eckstein N et al (2009) Hyperactivation of the insulin-like growth factor receptor I signaling pathway is an essential event for cisplatin resistance of ovarian cancer cells. Cancer Res 69:2996–3003. https://doi.org/10.1158/0008-5472.CAN-08-3153

Fabel K et al (2001) Long-term stabilization in patients with malignant glioma after treatment with liposomal doxorubicin. Cancer 92:1936–1942. https://doi.org/10.1002/1097-0142(20010100)92:7<3196::aid-cncr1712%3e3.0.co;2-h

Fanoe S, Jensen GB, Sjogren P, Korsgaard MP, Grunnet M (2009) Oxycodone is associated with dose-dependent QTc prolongation in patients and low-affinity inhibiting of hERG activity in vitro. Br J Clin Pharmacol 67:172–179. https://doi.org/10.1111/j.1365-2125.2008.03272.x

Fiorillo A et al (2004) Second-line chemotherapy with the association of liposomal daunorubicin, carboptatin and etoposide in children with recurrent malignant brain tumors. J Neurooncol 66:179–185. https://doi.org/10.1023/b:neon.000013471.53015.52

Friesen C, Roscher M, Alt A, Miltner E (2008) Methadone, commonly used as maintenance medication for outpatient treatment of opioid dependence, kills leukemia cells and overcomes chemoresistance. Cancer Res 68:6059–6064. https://doi.org/10.1158/0008-5472.CAN-08-1227

Friesen C et al (2013) Cell death sensitization of leukemia cells by opioid receptor activation. Oncotarget 4:677–690. https://doi.org/10.18632/oncotarget.952

Friesen C et al (2014) Opioid receptor activation triggering down-regulation of cAMP improves effectiveness of anti-cancer drugs in treatment of glioblastoma. Cell Cycle 13:1560–1570. https://doi.org/10.4161/cc.28493

Haas B et al (2009) Protein kinase G controls brown fat cell differentiation and mitochondrial biogenesis. Sci Signal 2:ra78. https://doi.org/10.1126/scisignal.2000511

Haas B et al (2018) Inhibition of the PI3K but not the MEK/ERK pathway sensitizes human glioma cells to alkylating drugs. Cancer Cell Int 18:69. https://doi.org/10.1186/s12935-018-0565-4

He Y, Kaina B (2019) Are there thresholds in glioblastoma cell death responses triggered by temozolomide? Int J Mol Sci 20:1562. https://doi.org/10.3390/ijms20071562

Insel PA, Zhang L, Murray F, Yokouchi H, Zambron AC (2012) Cyclic AMP is both a pro-apoptotic and anti-apoptotic second messenger. Acta Physiol (Oxf) 204:277–287. https://doi.org/10.1111/j.1748-1716.2011.02273.x

Inturrisi CE, Colburn WA, Kaiko RF, Houde RW, Foley KM (1987) Pharmacokinetics and pharmacodynamics of methadone in patients with chronic pain. Clin Pharmacol Ther 41:392–401. https://doi.org/10.1038/cpt.1987.47

Kaina B, Beltzig L, Piec-Staffa A, Haas B (2020) Cytotoxic and senolytic effects of methadone in combination with temozolomide in glioblastoma cells. Int J Mol Sci. https://doi.org/10.3390/ijms21197006

Katchman AN, McGroary KA, Kibhorn MJ, Kornick CA, Manfredi PL, Woosley RL, Ebert SN (2002) Influence of opioid agonists on cardiac human ether-a-go-go related gene K+-currents. J Pharmacol Exp Ther 303:688–694. https://doi.org/10.1124/jpet.102.038240

Krantz MJ, Mehler PS (2004) Treating opioid dependence. Growing implications for primary care. Arch Intern Med 164:277–288. https://doi.org/10.1001/archinte.164.3.277

Landgraf V, Griessmann M, Roller J, Polednik C, Schmidt M (2019) d,l-Methadone as an enhancer of chemotherapeutic drugs in head and neck cancer cell lines. Anticancer Res 39:3633–3639. https://doi.org/10.21877/anticanres.13511

Linares OA, Fudin J, Daly A, Schiesser WE, Boston RC (2015) Methadone recycling sustains drug reservoir in tissue. J Pain Palliat Care Pharmacother 29:261–271. https://doi.org/10.3109/1536288.2015.1047552

Louis DN et al (2016) The 2016 world health organization classification of tumors of the central nervous system: a summary.
Acta Neuropathol 131:803–820. https://doi.org/10.1007/s00401-016-1455-1

Merker PC, Lewis MR, Walker MD, Richardson EP Jr (1978) Neurotoxicity of adriamycin (doxorubicin) perfused through the cerebrospinal fluid spaces of the rhesus monkey. Toxicol Appl Pharmacol 44:191–205. https://doi.org/10.1016/0041-008x(78)90298-3

Michalska M, Schultz-Semmann S, Kuckuck I, Katzenwadel A, Wolf P (2018) Impact of methadone on cisplatin treatment of bladder cancer cells. Anticancer Res 38:1369–1375. https://doi.org/10.21873/anticancer.12360

Neuwelt EA, Pagel M, Barnett P, Glassberg M, Frenkel EP (1981) Pharmacology and toxicity of intracarotid adriamycin administration following osmotic blood-brain barrier modification. Cancer Res 41:4466–4470

Oldendorf WH, Hyman S, Braun L, Oldendorf SZ (1972) Blood–brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. Science 178:984–986. https://doi.org/10.1126/science.178.4064.984

Oppermann H et al (2019) l-Methadone does not improve radio- and chemotherapy in glioblastoma in vitro. Cancer Chemother Pharmacol 83:1017–1024. https://doi.org/10.1007/s00280-019-03816-3

Ostrom QT, Gittleman H, Xu J, Kroemer C, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS (2016) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. Neuro Oncol 18:v1–v75. https://doi.org/10.1093/neuonc/nov207

Parsons HA, de la Cruz M, El Osta B, Li Z, Calderon B, Palmer JL, Bruera E (2010) Methadone initiation and rotation in the outpatient setting for patients with cancer pain. Cancer 116:520–528. https://doi.org/10.1002/cncr.24754

Perez-Alvarez S et al (2010) Methadone induces necrotic-like cell death in SH-SY5Y cells by an impairment of mitochondrial ATP synthesis. Biochim Biophys Acta 1802:1036–1047. https://doi.org/10.1016/j.bbadis.2010.07.024

Perez-Alvarez S, Iglesias-Guimaraes V, Solesio ME, Melero-Fernandez de Mera RM, Yuste VJ, Galindo MF, Jordan J (2011) Methadone induces CAD degradation and AIF-mediated necrotic-like cell death in neuroblastoma cells. Pharmacol Res 63:352–360. https://doi.org/10.1016/j.phrs.2010.12.001

Roos W, Baumgartner M, Kaina B (2004) Apoptosis triggered by DNA damage O-6-methylguanine in human lymphocytes requires DNA replication and is mediated by p53 and Fas/CD95/Apo-1. Oncogene 23:359–367. https://doi.org/10.1038/sj.onc.1207080

Sales TT, Resende FFB, Chaves NL, Titze-De-Almeida SS, Bao SN, Brettas ML, Titze-De-Almeida R (2016) Suppression of the Eagl potassium channel sensitizes glioblastoma cells to injury caused by temozolomide. Oncol Lett 12:2581–2589. https://doi.org/10.3892/ol.2016.4992

Shi L et al (2019) Methadone enhances the effectiveness of 5-aminovulinic acid-based photodynamic therapy for squamous cell carcinoma and glioblastoma in vitro. J Biophotonics 12:e201800468. https://doi.org/10.1002/jbio.201800468

Shi L et al (2020) MOP-dependent enhancement of methadone on the effectiveness of ALA-PDT for A172 cells by upregulating phosphorylated JNK and BCL2. Photodiagn Photodyn 30:101657. https://doi.org/10.1016/j.pdpdt.2020.101657

Siegel RL, Miller KD, Jemal A (2020) Cancer statistics, 2020 CA Cancer J Clin 70:7–30. https://doi.org/10.3322/caac.21590

Singh A, Jayanthan A, Farran A, Elwi AN, Kim SW, Farran P, Narendran A (2011) Induction of apoptosis in pediatric acute lymphoblastic leukemia (ALL) cells by the therapeutic opioid methadone and effective synergy with Bcl-2 inhibition. Leukemia Res 35:1649–1657. https://doi.org/10.1016/j.leukres.2011.06.035

Stupp R et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987–996. https://doi.org/10.1056/NEJMoa043330

Stupp R et al (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459–466. https://doi.org/10.1016/S1470-2045(09)70025-7

Vatter T, Klumpp L, Ganzer K, Stransky N, Zips D, Eckert F, Huber SM (2020) Against repurposing methadone for glioblastoma therapy. Biomolecules. https://doi.org/10.3390/biom10060917

Wang XZ, Chen YF, Zhang YH, Guo S, Mo L, An HL, Zhan Y (2017) Eagl voltage-dependent potassium channels: structure, electrophysiological characteristics, and function in cancer. J Membr Biol 250:123–132. https://doi.org/10.1007/s00232-016-9944-8

Zunkler BJ, Wos-Maganga M (2010) Comparison of the effects of methadone and heroin on human ether-a-go-go-related gene channels. Cardiovasc Toxicol 10:161–165. https://doi.org/10.1007/s12012-010-0974-y

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