Cancer Chemotherapy and Pharmacology (2019) 83:1017–1024
https://doi.org/10.1007/s00280-019-03816-3

ORIGINAL ARTICLE

D,L-Methadone does not improve radio- and chemotherapy in glioblastoma in vitro

Henry Oppermann[1] · Martina Matusova[1] · Annegret Glasow[2] · Johannes Dietterle[1] · Rainer Baran-Schmidt[1] · Karsten Neumann[3] · Jürgen Meixensberger[1] · Frank Gaunitz[1]

Received: 19 November 2018 / Accepted: 13 March 2019 / Published online: 19 March 2019

Abstract

Purpose Glioblastoma (GBM) is the most common malignant tumor of the central nervous system. Median survival of glioblastoma patients under standard therapy including radiotherapy and chemotherapy using temozolomide (TMZ) is 14.6 months. As cell culture experiments combining D,L-methadone with doxorubicin demonstrated an increased reduction of cell viability of glioblastoma cells, the opioid has been discussed as a drug for the treatment of GBM. Despite lack of clinical and experimental evidence that D,L-methadone in combination with standard therapy will be beneficial, an increasing number of tumor patients medicating themselves with D,L-methadone present to the hospitals in Germany.

Methods As a first step towards understanding whether D,L-methadone may increase the efficacy of standard therapy, we used a cell culture model of primary GBM and fibroblast cell cultures derived from GBM patients. The cultures were treated with different concentrations of D,L-methadone in combination with X-irradiation, TMZ or both. Cell viability was determined by measuring ATP in cell lysates and dehydrogenase activity in living cells.

Results When only treated with D,L-methadone, 1 µM of the opioid was sufficient to reduce viability of fibroblasts, whereas 10 µM was needed to significantly reduce glioblastoma cell viability. In addition, D,L-methadone did not improve the anti-neoplastic effects of X-irradiation, temozolomide or both.

Conclusions As D,L-methadone reduces glioblastoma cell viability only when concentrations are used that had been reported to be toxic to patients and as there were no interactions observable combining it with standard therapy, a recommendation for the use of D,L-methadone in glioblastoma therapy cannot be given.

Keywords D,L-Methadone · Glioblastoma · Temozolomide · X-irradiation · Standard therapy

Henry Oppermann and Martina Matusova contributed equally.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00280-019-03816-3) contains supplementary material, which is available to authorized users.

✉ Frank Gaunitz
Frank.Gaunitz@medizin.uni-leipzig.de

Henry Oppermann
Henry.Oppermann@medizin.uni-leipzig.de

Martina Matusova
Martina.Matusova@medizin.uni-leipzig.de

Annegret Glasow
Annegret.Glasow@medizin.uni-leipzig.de

Johannes Dietterle
Johannes.Dietterle@medizin.uni-leipzig.de

Rainer Baran-Schmidt
Rainer.Baran-Schmidt@medizin.uni-leipzig.de

Karsten Neumann
Karsten.Neumann@klinikum-dessau.de

Jürgen Meixensberger
Juergen.Meixensberger@medizin.uni-leipzig.de

[1] Department of Neurosurgery, University Hospital Leipzig, Liebigstraße 20, 04103 Leipzig, Germany

[2] Department of Radiooncology, University of Leipzig, Leipzig, Germany

[3] Department of Pathology, Medical Clinic Dessau, Dessau, Germany

Introduction

The most common malignant primary tumor of the central nervous system is glioblastoma (GBM) [1]. Under standard therapy, consisting of tumor resection and adjuvant radio- and chemotherapy using temozolomide (TMZ), median survival of patients is 14.6 months [2]. After many years of research and clinical trials with new drugs or new treatment strategies only minor improvements have been made such as by combining standard therapy with locoregionally delivered antimitotics (tumor-treating fields; TTF), which increased median overall survival to 20.9 months compared to 16.0 months in a temozolomide-alone group [3]. Hence, there is an urgent need for new therapeutic strategies to treat glioblastoma. Understandably patients are also willing to try whatever appears to be of help for the treatment of their disease, even if the available data is poor and there is no scientific proof for the efficacy of the treatment. Such is the case with the treatment of glioblastoma by D,L-methadone—at least in Germany [4]. A significant number of patients suffering from glioblastoma (and already other types of tumors) requested or already started to use D,L-methadone after reports in the public press of Germany in 2017 [5] (see also data from a survey of the "Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie e. V." which is available online: https://www.dgho.de/publikationen/stellungnahmen/gute-aerztliche-praxis/methadon/Methadon_Ergebnisse_Mitgliederumfrage_20170824.pdf/view). Moreover, the public discussion about the beneficial effects of D,L-methadone already resulted in a petition to the German government asking for funds for clinical trials investigating the opioids' impact on cancer therapy [German government; petition number: 78411 (https://epetitionen.bundestag.de/petitionen/_2018/_04/_30/Petition_78411.nc.html)] which already reached quorum. The discussion was originally started after public reports based on observations of Friesen and co-workers who demonstrated an effect of D,L-methadone on two glioblastoma cell lines when combined with doxorubicin [6]. In the same manuscript the authors also report a reduced growth of U87 glioblastoma cells transplanted into nude mice that were treated with D,L-methadone alone. Although it appears to be safe to use D,L-methadone for therapy at least under the concentrations currently discussed for the treatment of cancer patients [7], there are no clinical data or even case reports available in the literature that unequivocally prove that D,L-methadone has beneficial effects in the treatment of glioblastoma. In addition, only few data are available with regard to a potential positive interaction between TMZ and D,L-methadone. Testing this possibility using four brain-tumor initiating cell lines (BITCs) and two established cell lines (U87 and U251), Brawansky et al. observed antiproliferative effects only at higher concentrations of D,L-methadone (four cell lines), no effect in one cell line and even significantly antagonistic effects in U87 [8]. Whether the inclusion of X-irradiation may change this picture has not been reported. Unfortunately, it is also not known, how non-tumor cells may respond to a combination of D,L-methadone with X-irradiation and TMZ. Before starting clinical trials with patients we considered that these studies should be done in advance. Therefore, we used cultures of tumor cells isolated from glioblastoma after surgery and fibroblast cultures established from galea from the same patients and subjected them to treatment with TMZ, X-irradiation and D,L-methadone in different combinations.

Materials and methods

Reagents

Unless stated otherwise, all chemicals were purchased from Sigma Aldrich (Taufkirchen, Germany).

Cell culture

Primary cell cultures were established as described before using tumor tissue or galea (for fibroblasts) obtained during standard surgery performed at the Neurosurgery Department of the University Hospital Leipzig [9]. All patients provided written informed consent according to the German laws, and in accordance with the 1964 Helsinki declaration and its later amendments, as confirmed by the local committee (#144-2008). In Table 1 all cultures used are depicted along with age and gender of the donor and the MGMT-promoter methylation status. Cell cultures were maintained in DMEM (4.5 g glucose/ml) supplemented with 2 mM Glutamax™, 1% penicillin/streptomycin (all from Thermo Fisher Scientific, Darmstadt, Germany) and 10% fetal bovine serum (Biochrom GmbH, Berlin, Germany) and cultivated in an incubator (37 °C, 5% CO_2). The MGMT-promoter methylation status of established cultures was determined by pyrosequencing using the PyroMark Q24 System and the PyroMark Q24 CpG MGMT kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. All cells used in the experiments were tested for the expression of nestin, which is supposed to be mainly present in the proliferating cell population [10]. In addition, we also tested for expression of GFAP, which is supposed to be negative in the proliferating cell population [11] (Supplemental Fig. 1).

Table 1 Cell cultures and tissue samples used in the study presented

Pat.	Age/years	Gender	Diagnosis	Label	Sample type	Pas.	MGMT methylation	GFAP	Nestin
1	69	Male	Glioblastoma wild type	P0493	Glioblastoma	4	3.00%	N	P
				P0495	Fibroblast	1	3.00%	n/a	n/a
2	41	Male	Glioblastoma wild type	P0514	Glioblastoma	8	3.60%	N	P
				P0516	Fibroblast	3	3.80%	n/a	n/a
3	75	Male	Glioblastoma wild type	P0556	Glioblastoma	6	3.60%	N	P
				P0557	Fibroblast	4	3.40%	n/a	n/a
4	73	Female	Glioblastoma wild type	P0560	Glioblastoma	3	14.60%	P	P
				P0562	Fibroblast	9	4.00%	n/a	n/a
5	73	Male	Glioblastoma wild type	P0622	Glioblastoma	4	3.60%	N	P
				P0623	Fibroblast	2	3.60%	n/a	n/a
6	74	Male	Glioblastoma wild type	P0627	Glioblastoma	6	3.20%	N	N
				P0628	Fibroblast	7	2.00%	n/a	n/a
7	60	Male	Glioblastoma wild type	P0023	Glioblastoma	54	96.40%	N	N
8	59	Female	Meningioma	P0744	Brain tissue	Tissue	n/a	n/a	n/a

The table indicates patients' age at surgery in years, gender, confirmed pathology, the internal label used for cultures and tissues, sample type and passage number of cell cultures used in the experiments. In addition, the methylation status of the MGMT promoter of the cultured cells is indicated and whether the culture exhibited cells expressing GFAP or Nestin. The label P0744 identifies tissue from a 59-year-old patient diagnosed with meningeoma. The brain tissue was derived from the peritumoral edema zone [not contrast enhanced in magnetic resonance imaging (MRI)] (note: this tissue had to be removed during surgery to get sufficient surgical access to the MRI contrast enhanced meningioma tumor tissue)

N no marker expression detectable, *P* marker expression detectable, *na* not investigated

Cell characterization by immunofluorescence

For nestin and glial fibrillary acidic protein (GFAP) detection, 7500 cells per well were seeded on 8-well chamber slides (Lab-Tek System 177445, Permanox; Thermo Fisher Scientific) and allowed to attach for 24 h. Then, cells were washed with phosphate buffered saline (PBS; Thermo Fisher Scientific), fixed in ethanol:acetone (1:2) for 10 min, permeabilized with 0.5% Triton-X100 for 5 min, and immersed in PBS with 10% goat serum and 0.25% Triton-X-100 for 30 min to block unspecific binding sites. Slides were then incubated at 4 °C overnight with antibodies against human nestin (anti-Nestin Antibody, clone10C2; MAB5326, Merck Millipore; 1:200) and against GFAP (GFAP (D1F4Q); #12389; Cell Signaling Technology, Frankfurt am Main, Germany; 1:200), diluted in PBS with 2% goat serum and 0.25% Triton-X100. After three washes with PBS, the slides were incubated at room temperature for 1 h with secondary antibodies (goat anti-mouse IgG F(ab′)2-Alexa488 and goat anti-rabbit IgG F(ab′)2-Alexa568; both Invitrogen; 1:1000) diluted in PBS with 2% goat serum and 0.25% Triton-X100. Nuclei were counterstained with DAPI (4′6-diamidino-2-phenylindole-dilactate 10 mg, 1:5.000; Invitrogen) for 5 min, and slides were mounted in Mowiol 4-88/DABCO (Sigma Aldrich). For controls, specific IgG isotypes were employed (Nestin: mouse IgG1, 1:100, Millipore; GFAP: rabbit Ig, 1:320, St. Cruz). ImageJ [12] was used to generate overlays of images obtained by microscopy.

Cell viability assays

For cell viability assays, cells were counted and seeded in 200 µl medium into sterile 96-well plates (µClear, Greiner Bio One, Frickenhausen, Germany) at a density of 400 cells per well. After 24 h of cultivation the medium was aspirated and fresh medium supplemented with or without 200 µM TMZ and/or D,L-methadone (0, 1 nM, 10 nM, 0.1 µM, 1 µM, 5 µM, 10 µM and 30 µM) was added. After 72 h cells were X-irradiated using a total dose of 4 Gy [13] (DARPAC 150-MC, RayTech; with a dose rate of 0.813 Gy/min). For control, cells were handled as if they had to be irradiated but were not exposed to X-rays. Two hours after irradiation, cells received fresh medium containing the supplements they had received before irradiation. After further cultivation for 70 h, cell viability was determined using the CellTiter-Glo Luminescent Cell Viability Assay (Promega, Mannheim, Germany) and the CellTiter-Blue Cell Viability Assay (Promega). All assays were carried out according to manufacturer's protocols. Luminescence and fluorescence were measured using a SpectraMax M5 multilabel reader (Molecular Devices, Biberach, Germany). Each condition was determined in sixfold by measuring six independent wells.

Western-blot analysis

Western blotting was performed as described previously [14]. Briefly, cells were detached from culture flasks or plates using StemPro Accutase (Thermo scientific) and collected by centrifugation. Cell pellets were lysed by sonification in ice cold RIPA buffer containing phosphatase and protease inhibitors. 30 µg of protein were separated on a 12% polyacrylamide gel, transferred to a low fluorescence PVDF membrane (ab133411 Abcam, Cambridge, United Kingdom) which afterwards was blocked and probed with an antibody against human mu-opioid-receptor (MOR) (AB1580-I, Merck Millipore, Darmstadt, Germany; 1:1000; 1 h) followed by secondary antibody (goat anti-rabbit-IRDye800 [LI-COR; 925-32211] 1:10 000; 1 h). Protein bands were visualized using an Odyssey Imaging System (LI-COR, Bad Homburg, Germany). For reference, an anti-GAPDH antibody was used (Cell Signaling; #2118 1:10000; 1 h).

Statistical analysis

Cell viability data were calculated as follows: each data point was normalized to the average signal of the untreated control (set to 100%) of each cell culture. Then, the median was calculated for each condition, following descriptive statistics with $N=6$ for each cell type (glioblastoma cells and fibroblasts) and each condition. When applicable, Welsh's t tests were performed using the algorithms implemented in Excel (Microsoft, Richmond, USA) (unpaired two-sample test with unequal variances). Multivariate analysis of variance was performed by using SPSS (IBM, New York, USA; Version: 24.0.0.2 64-bit). P values were adjusted according to Benjamini and Hochberg [15] and a value <0.05 was presumed to be significant.

Results

Expression of µ-opioid receptor in primary glioblastoma cells

As D,L-methadone is an agonist at the µ-opioid receptor (MOR), we investigated whether the receptor is expressed in the cell cultures considered for our experiments. Therefore, proteins isolated from six patient-derived glioblastoma cultures were subjected to Western blotting. As can be seen in Fig. 1a, all cultures aside from P0622 express the glycosylated receptor at > 85 (Glyco 1) [16]. The > 66 kDa peptide (Glyco 2) is only seen in the non-malignant brain tissue but some cells also exhibit additional isoforms that according to the supplier of the antibody are located at 55 (Iso 10), 45 (Iso 1), 36 (Iso 13) and 34 (Iso 12) kDa. Whether the other bands detected are simply unspecific or belong to other (glycosylated) forms is unknown. In conclusion, all glioblastoma cell cultures express MORs, although there is a high degree of variability with regard to the isoforms present. As there were indications from the literature that D,L-methadone dependent on the concentration used may decrease or increase expression of MOR mRNA [17], we investigated this possibility using the cell cultures P0493 and P0514 which strongly differed in expression of MOR (Fig. 1a). The cells were treated as described in the section "Cell viability assays" (Material and methods). They received 5 µM D,L-methadone alone (M), were X-irradiated in the absence of D,L-methadone (X; 4 Gy) or were treated by both conditions. MOR protein expression was compared to untreated control cells (C). As can be seen in Fig. 1b, MOR expression in culture P0514 was unaffected by treatment. In culture P0493, which exhibits a low expression of Glyco 1 when compared to P0514, both, D,L-methadone and X-irradiation, increased expression of this isoform, although no additive effect was detectable. In addition, Iso 10 and Iso 12 appear to be positively affected by D,L-methadone, but this result is suspicious as the effect appears to be lost when cells are exposed to X-irradiation in addition.

Viability of glioblastoma cells and fibroblasts under the influence of D,L-methadone in combination with X-irradiation and temozolomide treatment

Here, we investigated whether D,L-methadone may increase the efficacy of radiotherapy and chemotherapy with TMZ using 6 patient-derived glioblastoma cell cultures (Patient 1–6). In parallel, we also investigated the effect on fibroblasts isolated from the same 6 patients to identify possible side effects on non-malignant cells. All cells were treated with or without 200 µM TMZ, 4 Gy of X-irradiation and different concentrations of D,L-methadone (0, 1 nM, 10 nM, 0.1 µM, 1 µM, 5 µM, 10 µM and 30 µM). As effects of D,L-methadone were previously reported to be seen after 144 h of treatment [6], we also determined cell viability after 144 h. In Fig. 2 the results with concentrations above 1 µM are summarized (data from concentrations below 1 µM, that did not show significant effects on viability, were omitted for readability; however, all data are available in Supplemental Fig. 2a, b). As can be seen in Fig. 2, a concentration of 10 µM D,L-methadone causes a significant effect on viability of glioblastoma cells that did not receive additional treatment. When viability is already reduced by TMZ and/or irradiation (at 0 µM D,L-methadone) the opioid does not significantly diminish viability further at this concentration. Although we observed a significant reduction of viability at a concentration of 30 µM D,L-methadone, this reduction was seen in all cells independent from additional treatment. In order to analyze whether

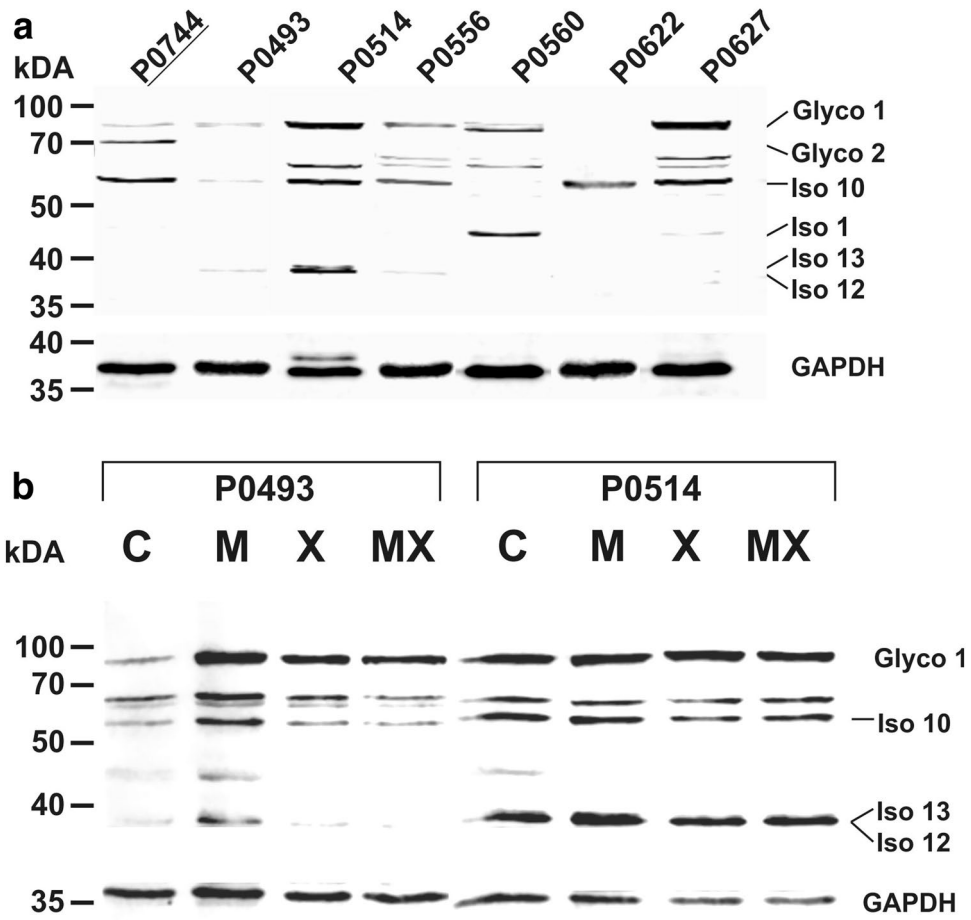

Fig. 1 MOR protein expression in patient-derived glioblastoma cultures. **a** Using an antibody directed against MOR isoforms 1, 10, 12 and 13, which also detects glycosylated isoforms, Western blot experiments were performed with protein from six patient-derived glioblastoma cell cultures. As a positive control for MOR expression, protein isolated from the tissue of the tumor-surrounding zone III from "Patient 8" (P0744, underlined) was used. GAPDH was used as loading control. The location of the 4 isoforms (Iso 1, Iso 10, Iso 12 Iso 13) and of the two glycosylated variants (Glyco 1, Glyco 2), that the antibody detects, are depicted to the right. **b** Primary glioblastoma cultures P0493 and P0514 were treated as described in "Material and methods" (section "Cell viability assays"). They received 5 µM D,L-methadone without X-irradiation (M), were subjected to a single dose of 4 Gy of X-irradiation (X) or treated by a combination of D,L-methadone and X-ray (MX). MOR protein expression of treated cells was then analyzed and compared to expression of untreated cells (C)

there are significant differences between the response of glioblastoma cells and fibroblasts, we performed a multivariate analysis with data obtained by both cell-based assays. This analysis revealed neither statistically significant difference ($p > 0.05$) between glioblastoma cells and fibroblasts with regard to standard therapy nor to their response to D,L-methadone or to the combination of D,L-methadone in combination with standard therapy. In addition, no effect of D,L-methadone on any kind of treatment was detected. From these calculations, we conclude that D,L-methadone does not increase the effect of TMZ and/or irradiation. This is also reflected by the simple observation that the reduction of viability of cells treated with 4 Gy, with TMZ or by both conditions remains unchanged with increasing concentrations of D,L-methadone (see Supplemental Fig. 3).

Discussion

Western blot experiments revealed expression of the µ-opioid receptor (MOR) in all of the patient-derived primary glioblastoma cell cultures. However, a significant effect on glioblastoma cell growth was not seen below a concentration of 10 µM D,L-methadone, which is in accordance with recently published data by Brawanski et al., who investigated the effect of D,L-methadone alone and in combination with TMZ in four BITCs and two established cell lines [8]. These authors reported a decrease in viability in the presence of D,L-methadone alone at concentrations between 15 and 45 µg/µL (~50–150 µM) and no reduction at a concentration of 1 µg/µL (3.2 µM) except for U87. In accordance with the experiments of Brawanski et al., we also did not detect an interaction between D,L-methadone and

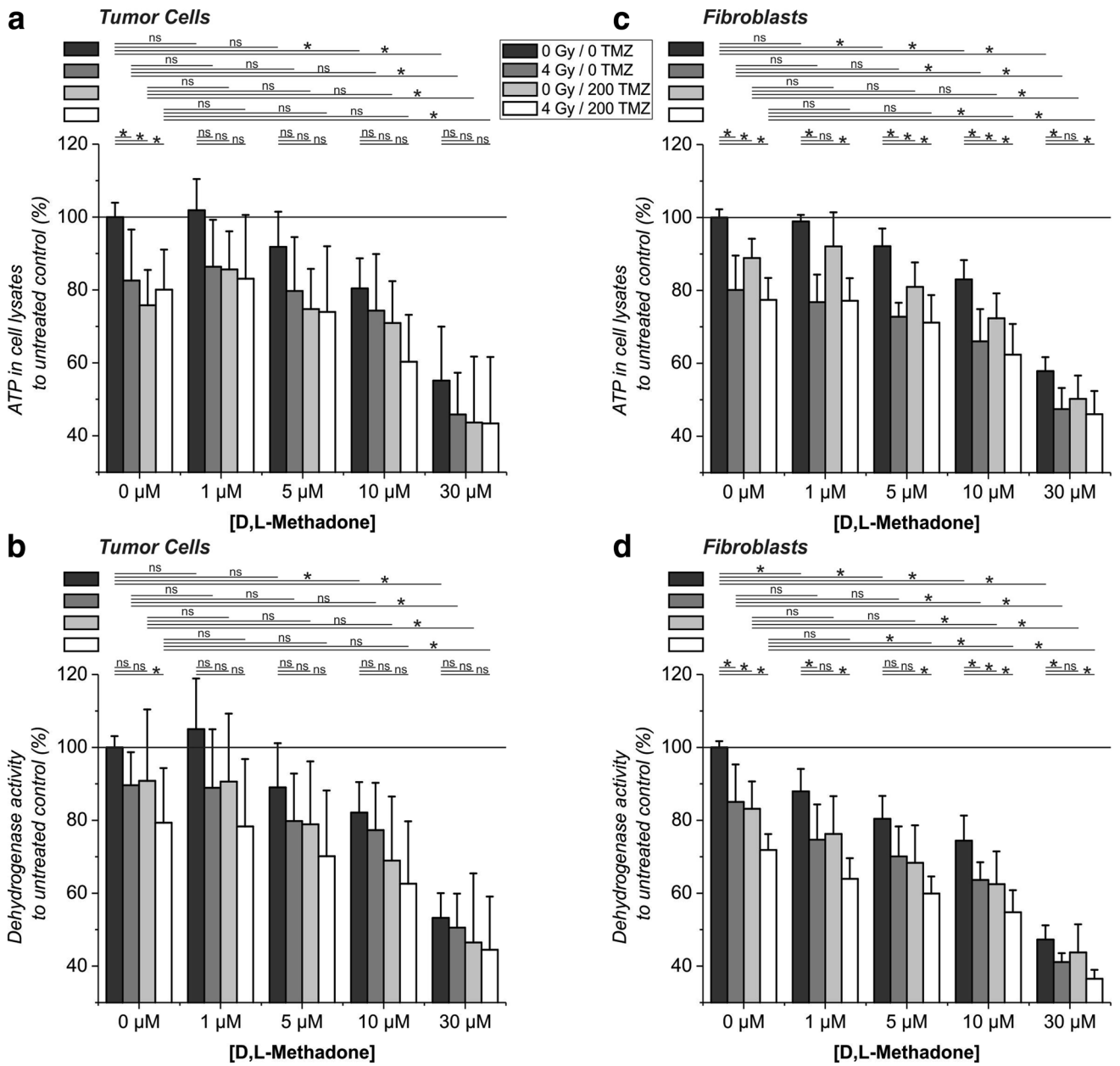

Fig. 2 Viability of patient-derived fibroblasts and glioblastoma cells under the influence of D,L-methadone, X-irradiation and TMZ. Glioblastoma cells (**a, b**) and fibroblasts cultures (**c, d**) were treated with different concentrations of D,L-methadone (1, 5, 10 or 30 µM) in combination with X-irradiation (4 Gy) and/or TMZ (200 µM). As a measure of viability the amount of ATP in cell lysates (**a, c**) and the activity of dehydrogenases in living cells (**b, d**) were determined. Bars represent the average and standard deviation of six glioblastoma and six fibroblast cultures (Patients 1–6) for each condition (0 Gy/0 TMZ, 4 Gy/0 TMZ, 0 Gy/200 TMZ and 4 Gy/200 TMZ; each represented by a different greyscale) and with different concentrations of D,L-methadone. Viability of cells that neither received D,L-methadone nor TMZ and were also not irradiated was set to 100 percent viability. Asterisks indicate statistical significance with adjusted p values according to Benjamini and Hochberg between different treatments indicated by the small bars above each panel (*$p < 0.05$; *ns* not significant)

TMZ at the concentrations employed in our experiments. In addition, we also did not observe an interaction with X-irradiation nor with a combination of X-irradiation and TMZ. Comparing the effect of D,L-methadone on fibroblasts as a model for non-tumor cells, we identified a loss of viability that was statistically indistinguishable to that seen in GBM cells under all conditions employed. At this point, it has to be noted, that fibroblasts are still able to proliferate and therefore do not reflect effects on non-proliferating neurons or glia cells. Unfortunately, patient-derived neurons or glia

cells are not available. Therefore, our experiments at least indicate, that non-tumor cells with the capacity to proliferate will be affected by treatment with D,L-methadone.

As stated above, we did not observe a significant effect on glioblastoma cell growth below a concentration of 10 µM D,L-methadone. At this point it is important to realize that concentrations above 10 µM can hardly be achieved at the side of the tumor. As Eap et al. pointed out, plasma concentrations of 400 µg/L for D,L-methadone (which is around 1.3 µM) might be used as target values in clinical management, requiring a final methadone dosage of at least 60 mg/day [18] (note: opioid-naïve patients receiving D,L-methadone typically start with an intravenous dose of 2.5–10 mg every 8–12 h which is gradually increased [19]). It should also be noted that toxicological analysis of adult deaths caused by methadone as the sole cause reported blood concentrations between 0.15 and 8.72 µM (median: 1.4 µM) [20]. In addition, Kreye and co-workers reported five case studies of clinical issues, rejecting the use of D,L-methadone as anti-tumor agent [21].

With regard to the cell culture experiments shown by Friesen et al. [6] one also has to note that this group combined D,L-methadone with doxorubicin. Although doxorubicin is used to treat different types of cancer including lung and breast cancer (for a review see [22]), this substance does not pass the blood–brain barrier. Whether combinations with other anti-cancer drugs may be beneficial is difficult to tell. Michalska for example observed an additive effect of D,L-methadone in combination with cisplatin in T24 but not in HT-1376 bladder carcinoma cells [23]. It is also difficult to score whether other tumors may in general be treatable by the opioid. Brüggen and co-workers for example could not detect an anti-neoplastic effect of D,L-methadone in melanoma [24], whereas Maneckjee and Minna observed a significant growth inhibiting effect in lung cancer cells with 10 nM of D,L-methadone [25].

As the cell cultures used in our experiments were MGMT-promoter methylation negative, one may also argue that patients with tumors with MGMT-positive promoter methylation may benefit from treatment. Aside from the fact that the cells employed in our experiment did respond to TMZ, we also tested one MGMT-promoter methylation positive glioblastoma culture (P0023) for which no fibroblast culture was available (Supplemental Fig. 4). Although this tumor had the expected much stronger response towards the treatment with TMZ, we again did not see any interaction of TMZ with D,L-methadone. Interestingly, we even identified a positive effect of D,L-methadone at lower concentrations. Although we do not know how to explain this phenomenon, it is interesting to note that this observation is in accordance with observations of Brawanski et al. who found a comparable effect in one of their glioblastoma cell lines investigated [8]. As we could see positive effects of D,L-methadone on viability at lower concentrations of the opioid in some cultures (Supplemental Figs. 2a, b), this raises the serious question whether some tumors may obtain a growth advantage under low concentrations of D,L-methadone. We think, that considering using D,L-methadone for the treatment of tumor patients, this phenomenon needs to be investigated in further detail before starting clinical trials.

Finally, it has to be noted, that the cell culture experiments performed in the study presented have the disadvantage that they cannot simulate the cells in their tissue-specific context. However, we currently have no other way to get initial data on whether D,L-methadone may be beneficial for treatment of glioblastoma patients. This could finally only be done by clinical studies that in our opinion cannot be supported by the currently available data from cell culture experiments. One may also argue that our cultures were kept in the presence of 10% bovine serum that after prolonged cultivation may change cell biology. At this point, we have to note, that we used early passages of cultures for the experiments that have been shown to convey reliable results in a number of our previous studies (e.g., [9, 26]). As demonstrated by nestin and GFAP staining (Supplemental Fig. 1), the cell cultures exhibit differences with regard to the expression of these markers. However, we believe that this is an advantage compared to using cell lines which may be better characterized, but do not reflect the heterogeneity of patients tumors. As there were no significant differences seen between the six different MGMT-promoter methylation negative tumor cell cultures with regard to treatment, this observation strongly supports that D,L-methadone cannot be expected to have beneficial effects on treatment.

Conclusion

As D,L-methadone reduces glioblastoma cell viability only when concentrations are used that had been reported to be toxic to patients and as there is no interaction between TMZ and/or irradiation, which are used in standard therapy, a recommendation for the use of D,L-methadone in glioblastoma therapy cannot be given.

Acknowledgements We like to thank Astrid Birnbaum (Department of Pathology, Medical Clinic Dessau) for determining the methylation of the MGMT promotor.

Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest in this work.

Informant consent All patients provided written informed consent according to German law as confirmed by the local committee (#144-2008) in accordance with the 1964 Helsinki declaration and its later amendments.

References

1. Ostrom QT, Gittleman H, Xu J et al (2016) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. Neuro-Oncology 18(suppl_5):v1–v75. https://doi.org/10.1093/neuonc/now207
2. Stupp R, Mason WP, van den Bent MJ et al (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996
3. Stupp R, Taillibert S, Kanner A et al (2017) Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. JAMA 318(23):2306–2316. https://doi.org/10.1001/jama.2017.18718
4. Hübner J, Hartmann M, Wedding U et al (2017) Methadon in der Onkologie: "Strohhalmfunktion" ohne Evidenz. Dtsch Arztebl International 114(33–34):A–A1530
5. Winkler E (2017) Methadon gegen Krebs—auch eine Frage für die Ethik und Theorie der Medizin. Ethik Med 29(4):269–272. https://doi.org/10.1007/s00481-017-0460-x
6. Friesen C, Hormann I, Roscher M et al (2014) Opioid receptor activation triggering downregulation of cAMP improves effectiveness of anti-cancer drugs in treatment of glioblastoma. Cell Cycle 13(10):1560–1570. https://doi.org/10.4161/cc.28493
7. Onken J, Friesen C, Vajkoczy P et al (2017) Safety and tolerance of D,L-methadone in combination with chemotherapy in patients with glioma. Anticancer Res 37(3):1227–1235. https://doi.org/10.21873/anticanres.11438
8. Brawanski K, Brockhoff G, Hau P et al (2018) Efficacy of D,L-methadone in the treatment of glioblastoma in vitro. CNS Oncol. https://doi.org/10.2217/cns-2018-0006
9. Oppermann H, Dietterle J, Purcz K et al (2018) Carnosine selectively inhibits migration of IDH-wildtype glioblastoma cells in a co-culture model with fibroblasts. Cancer Cell Int 18:111. https://doi.org/10.1186/s12935-018-0611-2
10. Arai H, Ikota H, Sugawara K-i et al (2012) Nestin expression in brain tumors: its utility for pathological diagnosis and correlation with the prognosis of high-grade gliomas. Brain Tumor Pathol 29(3):160–167. https://doi.org/10.1007/s10014-012-0081-5
11. Schiffer D, Giordana MT, Germano I et al (1986) Anaplasia and heterogeneity of GFAP expression in gliomas. Tumori 72(2):163–170
12. Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675
13. Merz F, Gaunitz F, Dehghani F et al (2013) Organotypic slice cultures of human glioblastoma reveal different susceptibilities to treatments. Neuro-Oncology 15(6):670–681. https://doi.org/10.1093/neuonc/not003
14. Oppermann H, Alvanos A, Seidel C et al (2018) Carnosine influences transcription via epigenetic regulation as demonstrated by enhanced histone acetylation of the pyruvate dehydrogenase kinase 4 promoter in glioblastoma cells. Amino Acids. https://doi.org/10.1007/s00726-018-2619-2
15. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodol) 57(1):289–300
16. Ferrer-Alcón M, La Harpe R, García-Sevilla JA (2004) Decreased immunodensities of micro-opioid receptors, receptor kinases GRK 2/6 and beta-arrestin-2 in postmortem brains of opiate addicts. Brain Res Mol Brain Res 121(1–2):114–122. https://doi.org/10.1016/j.molbrainres.2003.11.009
17. Saify K, Saadat M (2016) Expression levels of OPRM1 and PDYN in human SH-SY5Y cells treated with morphine and methadone. Life Sci 150:39–41. https://doi.org/10.1016/j.lfs.2016.02.078
18. Eap CB, Buclin T, Baumann P (2002) Interindividual variability of the clinical pharmacokinetics of methadone: implications for the treatment of opioid dependence. Clin Pharmacokinet 41(14):1153–1193. https://doi.org/10.2165/00003088-200241140-00003
19. Lugo RA, Satterfield KL, Kern SE (2005) Pharmacokinetics of methadone. J Pain Palliat Care Pharmacother 19(4):13–24. https://doi.org/10.1080/J354v19n04_05
20. Milroy CM (2000) Methadone deaths: a toxicological analysis. J Clin Pathol 53(4):277–281. https://doi.org/10.1136/jcp.53.4.277
21. Kreye G, Masel E-K, Hackner K et al (2018) Methadon als antitumortherapie: Schwindel, Hoffnung oder Risiko?: Eine Serie von Kasuistiken und kurzer Überblick über aktuelle Literatur sowie Empfehlungen der Fachgesellschaften (Methadone as anticancer treatment: hype, hope, or hazard?: a series of case reports and a short review of the current literature and recommendations of the societies). Wien Med Wochenschr 168(7–8):159–167. https://doi.org/10.1007/s10354-018-0623-5
22. Tacar O, Sriamornsak P, Dass CR (2013) Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 65(2):157–170. https://doi.org/10.1111/j.2042-7158.2012.01567.x
23. Michalska M, Schultze-Seemann S, Kuckuck I et al (2018) Impact of methadone on cisplatin treatment of bladder cancer cells. Anticancer Res 38(3):1369–1375. https://doi.org/10.21873/anticanres.12360
24. Brüggen M-C, Mangana J, Irmisch A et al (2018) Methadone-Not a magic bullet in melanoma therapy. Exp Dermatol 27(6):694–696. https://doi.org/10.1111/exd.13543
25. Maneckjee R, Minna JD (1992) Nonconventional opioid binding sites mediate growth inhibitory effects of methadone on human lung cancer cells. Proc Natl Acad Sci USA 89(4):1169–1173
26. Renner C, Seyffarth A, Arriba S de et al (2008) Carnosine inhibits growth of cells isolated from human glioblastoma multiforme. Int J Pept Res Ther 14:127–135. https://doi.org/10.1007/s10989-007-9121-0