

# Chemotherapy Sensitizing Effects of Methadone in Glioblastoma Cells Are Drug- and Cell Line-dependent

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## Primary research

**Keywords:** Glioblastoma, drug resistance, PI3K, temozolomide, cisplatin

**Posted Date:** August 21st, 2020

**DOI:** <https://doi.org/10.21203/rs.3.rs-62227/v1>

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# Abstract

## Background

D,L-methadone (MET), an analgesic drug used for pain treatment and opiate addiction has achieved attention from oncologist and social media as possible chemosensitizing agent in glioblastoma multiforme (GBM) treatment. MET has been reported to enhance doxorubicin-induced cytotoxicity in GBM cells via activation of the  $\mu$ -opioid receptor (MOR) and subsequent apoptosis induction. Here, we further aimed at quantifying MET effects in comparison to other opioids alone and in combination with doxorubicin and clinically more relevant temozolomide (TMZ) in 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 or in combination was assessed by MTT assays. Concentration effect curves were fitted to data points by nonlinear regression analysis and  $IC_{50}$  values were calculated. Apoptotic rates were determined by Annexin V staining.

## Results

We found that MET alone was cytotoxic to GBM cells at high micromolar concentrations in MTT assays by induction of apoptosis and necrosis while morphine and oxycodone were hardly cytotoxic. 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 by MTT assays in combination with fixed MET concentrations. MET only enhanced doxorubicin cytotoxicity in one cell line and in part in primary cells at certain MET concentrations. MET was not effective in sensitizing cells towards TMZ. Contrarily, in two cell lines MET even decreased sensitivity towards TMZ.

## Conclusions

MET can be considered cytotoxic to GBM cells only at clinically not relevant concentrations by induction of apoptosis and necrosis. Sensitizing effects are only observed in combination with doxorubicin but not with TMZ and are highly dependent on cell line and applied drug concentrations.

## Background

Grade IV glioblastoma multiforme (GBM) is the most aggressive form of glioma with highest incidence [1, 2]. Median survival is 14.6 months under therapy, the 5-year survival rate only is at 6% [3], indicating that there is high medical need for new therapeutic options. Therapeutic standard of care in GBM treatment is

radiotherapy with concomitant temozolomide (TMZ) treatment [4, 5]. Recent studies indicate that D,L-methadone (MET), an analgesic drug used for pain treatment and opiate addiction [6, 7], increases apoptosis of leukemia cells and the cytotoxic effects of the topoisomerase II-inhibitor doxorubicin. In a follow up study, the same group investigated MET on GBM cell lines and showed that the opioid has the potential to enhance apoptosis induced by doxorubicin [8–10]. The proposed mechanism of action is activation of the  $\mu$ -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 [11] raising the question if all GBM cells respond equally to MET treatment. In addition, it has been shown that MET increases intracellular doxorubicin levels probably by inhibiting P-glycoproteins (P-gps) in GBM cells. In a tumor model of nude mice implanted with U87 GBM cells, MET treatment reduced tumor growth and volume [9]. On the other hand, more recent data indicate no sensitizing effect of MET on various doxorubicin-treated canine tumor cells [12].

The first-line therapy in GBM treatment is TMZ in combination with radiotherapy, while doxorubicin in first place has no indication for GBM and second is rarely used off-label due to its poor blood-brain barrier penetration and neurologic side effects [13, 14]. Despite 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. Current in vitro studies did not report a potentiation of TMZ effects in GBM cells [15, 16], questioning the use of MET in GBM (reviewed in [17]).

Therefore, we aimed at investigating the quantitative contribution of MET to doxorubicin- and TMZ-induced cytotoxicity in established GBM cell lines and primary GBM 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 in a panel of cells to quantify effects and determine  $IC_{50}$  values. Furthermore, we applied the MOR inhibitor naloxone and opioids used in oncology, morphine and oxycodone, to proof if opioid-induced cytotoxicity is mediated via MOR. Although MET was cytotoxic itself at high, clinically not relevant concentrations in all GBM cells, we only observed weak MET-induced sensitization of one cell line and primary cells to doxorubicin, while there was no effect of MET on TMZ-induced cell death.

## Methods

## Materials

Temozolomide (Temodal®), doxorubicin hydrochloride, D,L-methadone hydrochloride, morphine sulfate salt pentahydrate, oxycodone, naloxone hydrochloride dihydrate, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO, USA ).

## 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). Primary GBM cells derived from a primary GBM tumor biopsy were obtained from the University Hospital Cologne, genetically characterized and cultured as previously described [18].

## 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 [19]. Anti-MOR-1 (D-12; 1:1000, Santa Cruz Biotechnology, TX, USA) combined with goat anti-mouse 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 [20]. Briefly, 5000 (U251, U87, U373) or 15,000 (primary) cells were plated on 96 wells and grown at 37 °C and 5% CO<sub>2</sub> 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. Final DMSO concentrations in media did not exceed 1%.

## Annexin V 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 × 10<sup>5</sup> cells were seeded into 6-well plates and incubated at 37° C and 5% CO<sub>2</sub> 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 propidium iodide 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 (FACSCalibur™, BD Bioscience, Franklin Lakes, NJ, USA).

## Fluorescence Microscopy

75,000 cells/mL were seeded on cover-glasses and incubated at 37° C and 5% CO<sub>2</sub> for 48 h before staining. Thereafter, cells were washed 2 times for 1 min with PBS and fixed with 4% paraformaldehyde/PBS for 10 min. Fixed cells were washed for 3 times with PBS and blocked in 2% bovine serum albumin (BSA; Sigma-Aldrich, St. Louis, MO, USA) including 0.2% Triton X-100 for 30 min. Thereafter cells were incubated with MOR-1- antibody (D12, Santa Cruz Biotechnology, TX, USA) in 2% BSA (1:50) at 4° C overnight. The next day cells were washed incubated with Anti-mouse IgG (H + L) DyLight™ 680 Conjugate (Cell Signalling Technology, Danvers, MA, USA) in 2% BSA (1:500) for 1 h. Finally, the stained cells were washed for 3 times with PBS and covered with a coverslip by using 1 drop of mounting medium. Only with secondary antibody stained cells, were used as negative control. MOR expression was visualized by using a Zeiss LSM 780 microscope (Carl Zeiss AG, Oberkochen, Germany) and 40 × magnification.

## 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). Statistical differences between two groups were determined by unpaired 2-tailed Student's *t*-test. Comparisons among several groups were performed by ANOVA followed by Turkey post-hoc test. Data are presented as means ± SEM

## Results

It has previously been shown that the MOR is expressed in GBM cell lines and primary cells [9, 15]. Here, we demonstrate 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 that MOR is expressed in a wide range of GBM cells to a similar extend.

We next asked whether MET is cytotoxic in these cells in MTT experiments including the A172 cell line in which beneficial effects of MET on doxorubicin-induced cytotoxicity has been reported [9]. We obtained very similar IC<sub>50</sub> values in all GBM cells in the range from 62 to 130 μM after 72-hour treatment. MET displayed a steep concentration response in all cells (Hill slopes ranging from - 3.3 to -4.4). The lowest IC<sub>50</sub> of 62 μM was obtained for A172 cells (Fig. 2a). Annexin V/propidium iodide staining of cell lines treated with the previously determined IC<sub>50</sub> MET concentrations revealed that approximately 50% of cell deaths account for apoptosis and necrosis, respectively (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 was not capable of abolishing MET-induced cytotoxicity (Fig. 2c-e). Contrarily, naloxone even significantly increased MET toxicity in A172 and primary cells (Fig. 2f), indicating that MET does not require the MOR for its cytotoxic action.

To further proof 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 a subset of GBM cell lines and primary cells (Fig. 3a-

d). Determined  $IC_{50}$  values were in the millimolar range or could not be established due to low cytotoxicity (Fig. 3e).

Friesen et al have previously shown that various MET concentrations (e.g. 10, 3, 1  $\mu\text{g}/\text{mL}$  corresponding to  $\sim 30, 10, 3 \mu\text{M}$ , respectively) were capable of sensitizing A172 cells towards a fixed doxorubicin concentration (0.3  $\mu\text{g}/\text{mL} = 0.5 \mu\text{M}$ ) in apoptosis assays. In order to determine a concentration relationship, we treated cells with ascending doxorubicin concentrations combined with 10  $\mu\text{M}$  MET, a concentration which itself is not cytotoxic but close to what might be reached in plasma of patients. MTT concentration-response curves overlapped in both tested GBM cell lines and in primary GBM cells (Fig. 4a-c); respective  $IC_{50}$  values showing no significant differences are displayed in Fig. 4d. However, it has to be noted that in A172 cells at 0.3  $\mu\text{M}$  doxorubicin, combination treatment with 10  $\mu\text{M}$  MET reduced cell viability as compared to doxorubicin treated cells alone (red circle in Fig. 4a). Interestingly, this is close to the concentrations tested by Friesen et al. (0.5  $\mu\text{M}$  doxorubicin combined with 3–30  $\mu\text{M}$  MET) where effects on apoptosis were observed.

Next, we repeated this experiment with previously determined  $IC_{50}$  concentrations of MET (Fig. 2a). High dose MET treatment (60  $\mu\text{M}$ ) 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  $\mu\text{M}$  MET (Fig. 5b). Also in primary GBM cells curves largely overlapped independently of treatment (Fig. 5c). Statistical analysis revealed that MET treatment significantly reduced doxorubicin  $IC_{50}$  values in A172 and primary GBM cells indicating a sensitization (Fig. 5d). Looking closer at the curves of primary GBM cells reveals that both curves overlap at most data points except at 1  $\mu\text{M}$  ( $\sim 0.5 \mu\text{g}/\text{mL}$ ) doxorubicin (red circle in Fig. 5c), which explains the difference in  $IC_{50}$  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 treatment option. We treated four GBM cell lines and primary GBM cells with ascending TMZ concentrations in combination with 10  $\mu\text{M}$  MET and performed MTT assays. In A172 cells MET even reduced sensitivity towards TMZ (Fig. 6a) while in all other cells no effect was observed (Fig. 6b-d). The only statistical difference in  $IC_{50}$  values was obtained for A172 cells (Fig. 6e) confirming the negative impact of MET on TMZ treatment in this cell line. Similar results were obtained when we treated cells with an  $IC_{50}$  MET concentration in combination with TMZ (Fig. 7). At this high MET concentrations both, A172 and U373 cell lines, respectively, were less sensitive 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 results of in vitro studies and xenograft mouse models [8–10]. In these studies, fixed doxorubicin concentrations combined with MET were applied to a subset of GBM cell lines and primary GBM cells and at tested concentrations MET was

capable of sensitizing these cells to doxorubicin in terms of apoptosis induction. The proposed mechanism of action is activation of MOR and subsequent induction of apoptosis in addition to P-gp inhibition by MET leading to increased intracellular doxorubicin levels [10]. In order to quantify whether MET alone is cytotoxic in GBM cells, we performed MTT assays with various MET concentrations. MET was indeed cytotoxic in a similar concentration range in various cell lines and primary cells. Yet, recorded  $IC_{50}$  values were far beyond concentrations, which might be reached in plasma of patients [21]. We also found that high dose MET induced apoptosis and necrosis to a similar extent in most cell lines except for U373 cells where about two thirds of recorded cell deaths accounted for necrosis. This is in line with what has been shown by others that MET can induce apoptosis [10] or necrotic-like cell death in cells of neuronal origin [22, 23] depending on cell type.

Interestingly, naloxone was not able to block MET-induced cytotoxicity and other MOR agonists, morphine and oxycodone, were hardly cytotoxic at clinically relevant concentrations. This implies that MET's cytotoxic action on GBM cells is not MOR dependent. MET might interfere with other cellular targets which are responsible for its cytotoxicity. For instance, MET, in contrast to oxycodone and morphine, also inhibits members of the voltage-gated potassium channel family at low micromolar concentrations [24–26]. Pharmacological inhibition of these channels in turn leads to cell death in various tumor cells including GBM cell lines [27] and is proposed as promising target for cancer treatment [28]. However, whether MET indeed blocks voltage-gated potassium channel at the applied concentrations in GBM cells leading to cytotoxicity needs further investigation. When we tested MET in combination with doxorubicin in a panel of GBM cell lines and primary cells we found a sensitizing effect only for the A172 cell line and to some extent in primary GBM cells. We have previously shown that P-gp is not expressed in U87, U251 and U373 cell lines [18] which might explain why these cells could not be sensitized to doxorubicin by MET, while inhibition of doxorubicin efflux has previously been reported for the A172 cell line [10]. Our findings are in line with a study which also reported no sensitizing effect of MET on various doxorubicin-treated canine tumor cells [12].

Another important finding of our study is, that the sensitizing effect of MET in A172 and primary cells highly depends on applied drug concentrations. While a complete left shift of the doxorubicin concentration-response curve upon MET treatment only was observed at high, clinically not relevant MET concentrations (60  $\mu$ M) in A172 cells, lower MET concentrations (10  $\mu$ M) 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 cytotoxic effects might occur. In addition, tolerable MET plasma levels in addicts are maximally 0.6  $\mu$ g/mL (corresponding to  $\sim$  2  $\mu$ M) after a dose of 60–120 mg/day [21, 29] which is much lower compared to effective MET concentrations reported by Friesen [10] and in our present study. When a blood-brain barrier penetration of only 42% for MET is assumed [30], 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 [31], however, if sufficient MET levels are indeed reached in brain tumor tissue at clinically achievable doses warrants

further investigation. Apart from that, doxorubicin is not the indicated treatment option for GBM due to its low blood-brain barrier penetration and neurological side effects [13, 14]. 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 in clinical practice. Due to these shortcomings, we also tested the clinically more relevant drug TMZ in combination with MET. One study has already reported no effect of MET on TMZ-induced cytotoxicity in various GBM cell lines [15]. Similar observations were made in another study with GBM cells and MET applied together with TMZ or irradiation (4 Gy) [16]. We could confirm these findings and most importantly in U373 and A172 cells, MET even reduced sensitivity towards TMZ.

## Conclusion

We conclude that cytostatic effects of MET alone on GBM cells are not mediated via the opioid receptor MOR implying that other cellular targets are involved what warrants further investigation. Furthermore, our findings do not support the use of MET in treatment of GBM in combination with TMZ as no sensitizing effects of MET were observed which is also in line what has been reported by others. The use of doxorubicin in GBM therapy is questionable and we doubt that feasible MET concentrations might be reached in brain of patients to achieve potential synergistic effects with doxorubicin, which we observed in vitro only in two distinct cell types at dedicated drug concentrations.

## Abbreviations

DOXO, doxorubicin; GBM, glioblastoma multiforme; Gi, inhibitory G-protein; MET, D,L-methadone; MOR,  $\mu$ -opioid receptor; NAL, naloxone; OXY, oxycodone; P-gp, P-glycoprotein; PKA, cAMP-dependent protein kinase; TMZ, temozolomide

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

## Funding

This article was supported by an intramural funding of the Federal Institute for Drugs and Medical Devices (BfArM).

## Authors' contributions

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

## Acknowledgment

We thank Marco Timmer for providing primary GBM cells.

## Authors' information

Disclaimer: The opinions mentioned throughout the following article are personal views of the authors and do not reflect an official position of the Federal Institute for Drugs and Medical Devices or an EMA-committee or working party, respectively.

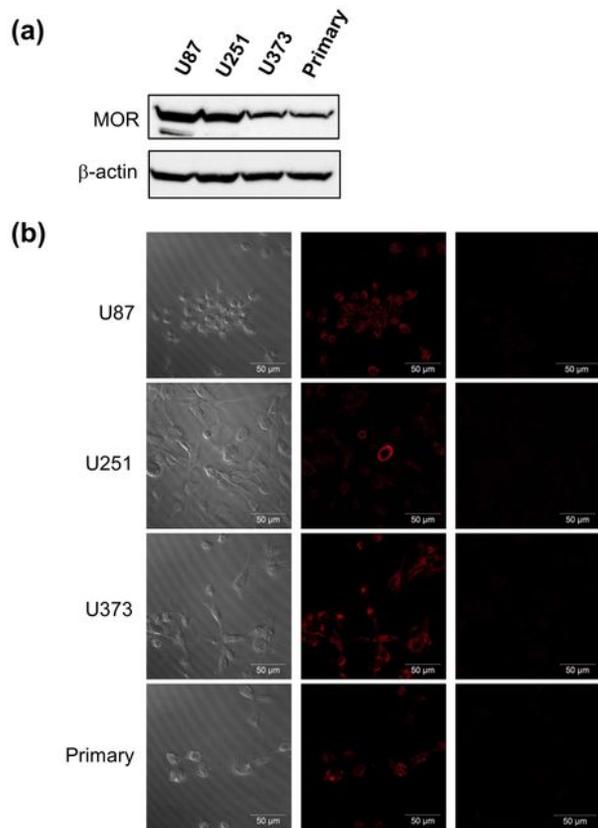
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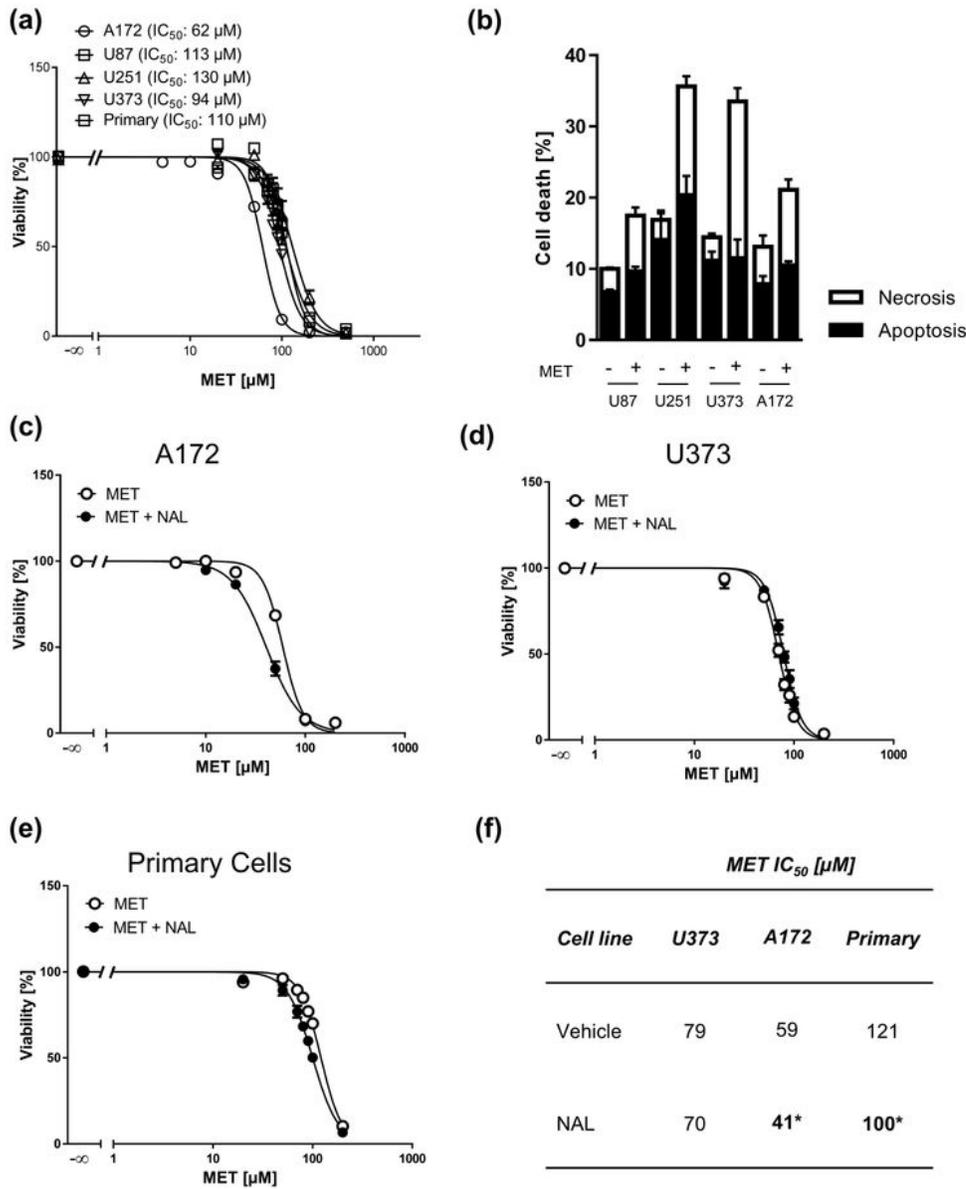
## Figures



**Figure 1**

$\mu$ -opioid receptor (MOR) expression analysis in human glioblastoma multiforme (GBM) cells. (a) Representative Western blot showing protein expression of MOR in U87, U251, U373 GBM cell lines and primary cells.  $\beta$ -actin Western blots were performed to control for loading. (b) Representative immune fluorescence images showing expression of MOR in U87, U251, U373 GBM cell lines and primary cells

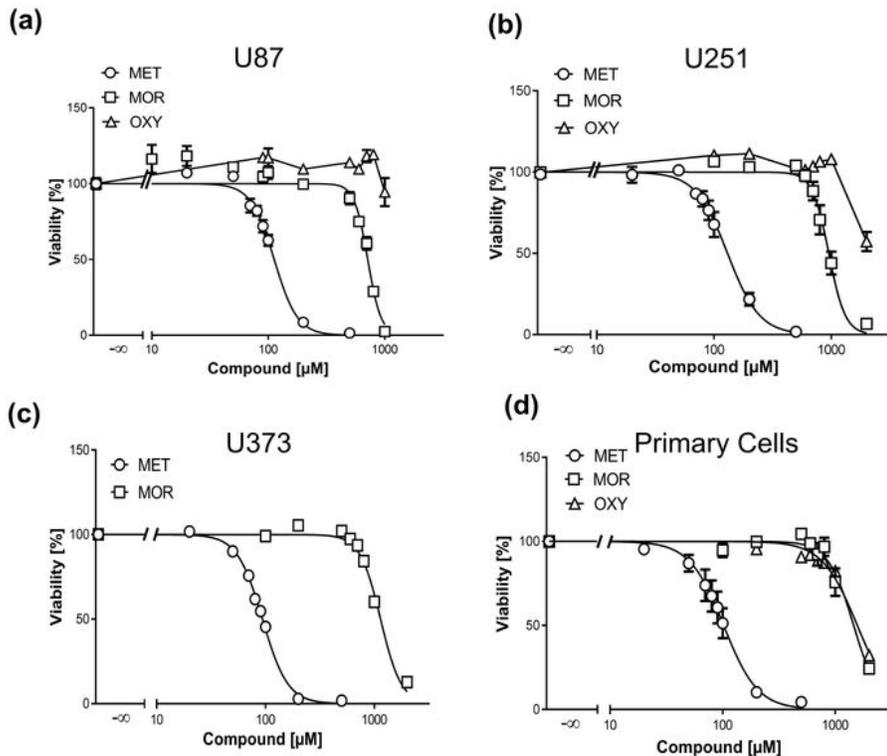
(middle panel). Left panels display bright field images of respective cells and right panels display cells treated with secondary antibody alone (negative control). Scale bar, 50  $\mu\text{m}$ .



**Figure 2**

Cytotoxicity of methadone (MET) in GBM cells. (a) 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays of four GBM cell lines and primary cells treated with increasing concentrations of MET for 72 h as indicated. (b) AnnexinV/propidium iodide (PI) FACS analysis of GBM cell lines U87, U251,

U373 and A172 treated with an IC50 MET concentration for 72 h. Annexin V positive cells were considered apoptotic and PI positive/Annexin V negative cells necrotic. Values are displayed as mean  $\pm$  SEM (n = 3-4). MTT assays of A172 (c), U373 (d)GBM cell lines and primary cells (e)treated with increasing concentrations of MET for 72 h pretreated with 300  $\mu$ M NAL for 1 h. Values are displayed as mean  $\pm$  SEM (n = 3-4). (f)IC50 values of MET in GBM cells derived from MTT assays; \*, p < 0.05.7



(e)

Cell line	U87	U251	U373	Primary
MET, $\mu$ M	113	130	94	102
MOR, $\mu$ M	714	953	1140	1458
OXY, $\mu$ M	N.C.	N.C.	-	1570

Figure 3

Comparison of opioid-induced cytotoxicity in GBM cell lines and primary cells. (a) MTT assays of U87, U251, U373 GBM cell lines and primary cells treated with increasing concentrations of MET, morphine (MOR) or oxycodone (OXY) for 72 h as indicated. Values are displayed as mean  $\pm$  SEM (n = 3-4). (b) IC<sub>50</sub> values of MET, MOR and OXY in GBM cells derived from MTT assays (a), N.C. = not calculated; - = not determined.

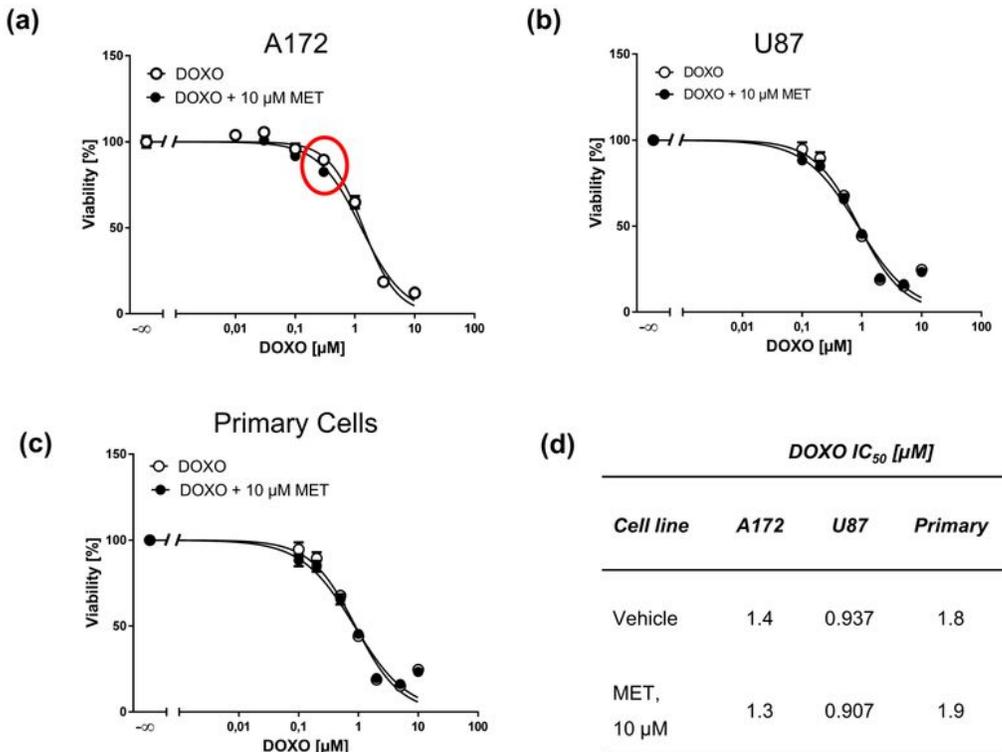


Figure 4

MET (10  $\mu\text{M}$ ) does not sensitize GBM cell lines and primary cells towards 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  $\mu\text{M}$  MET for 1 h. (d) IC<sub>50</sub> values derived from MTT assays of doxorubicin treated GBM cells with or without 10  $\mu\text{M}$  MET. Values are displayed as mean  $\pm$  SEM (n = 3). Note: in A172 at one data point combination treatment differed from DOXO treatment (0.3  $\mu\text{M}$ , red circle in (a)).

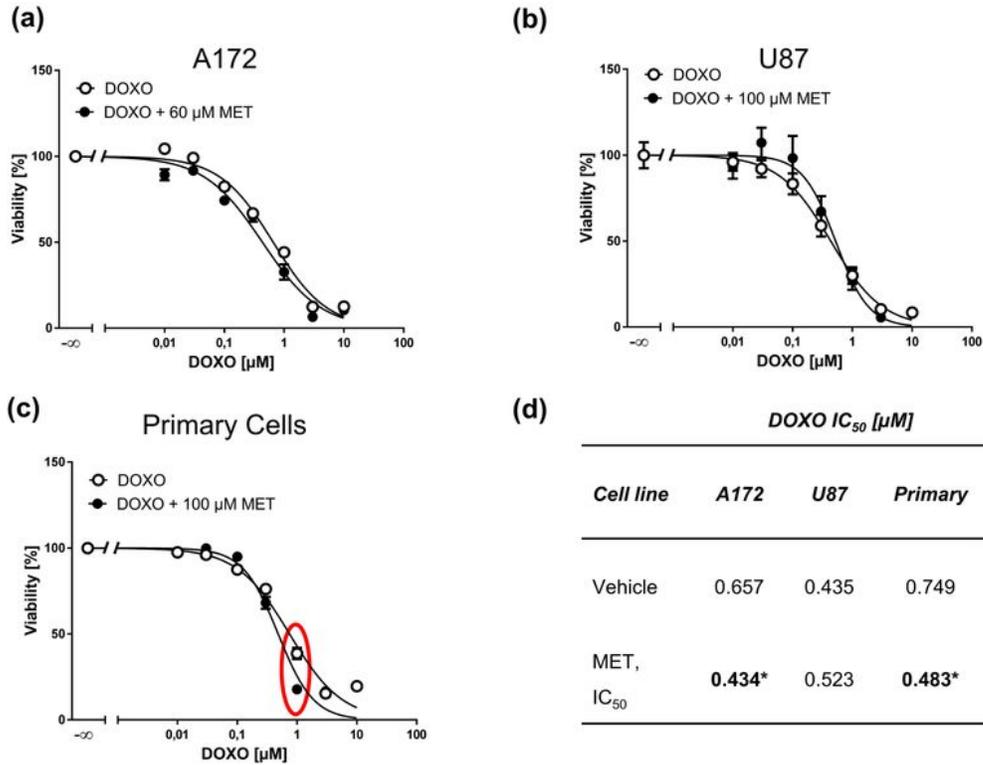
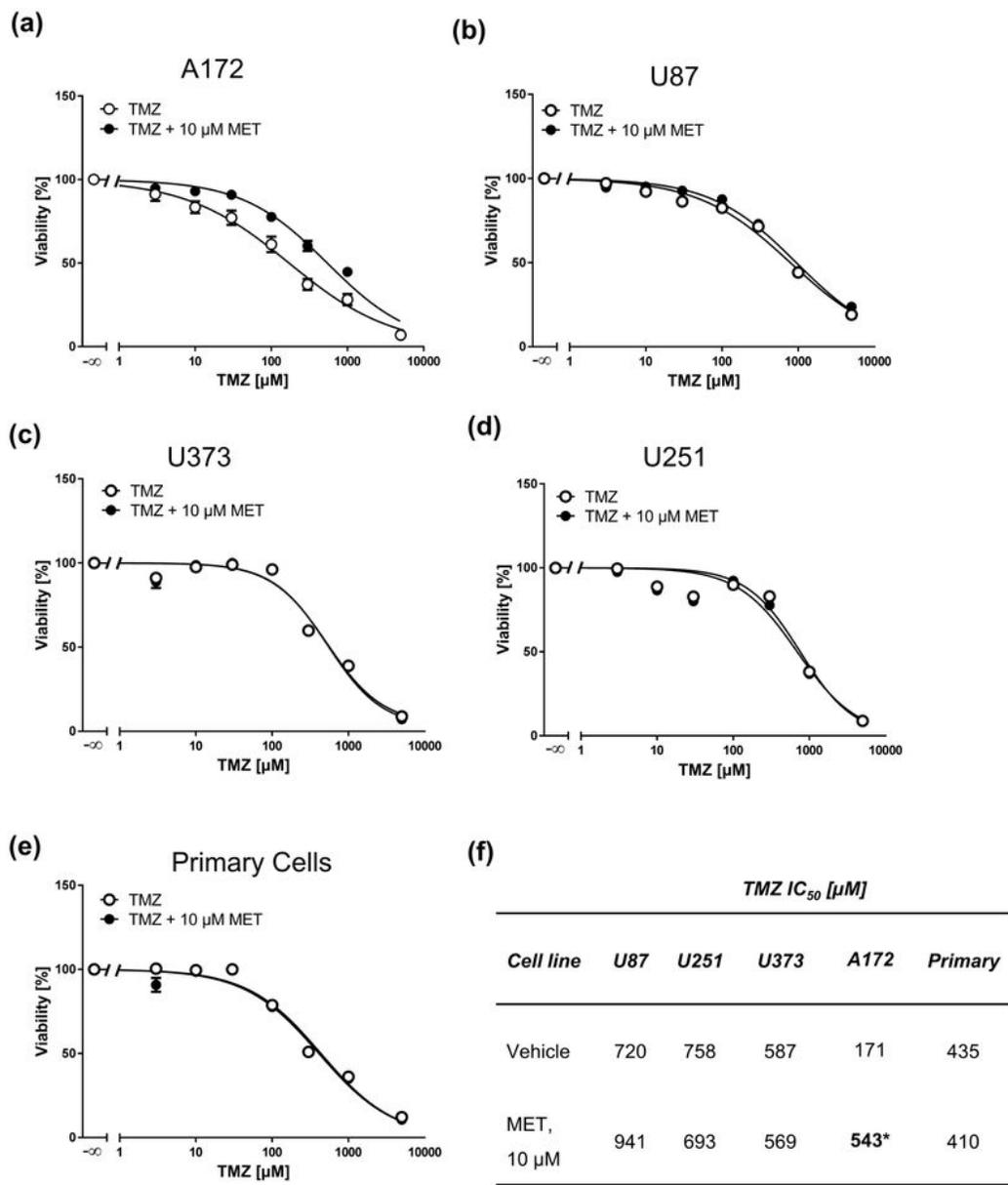


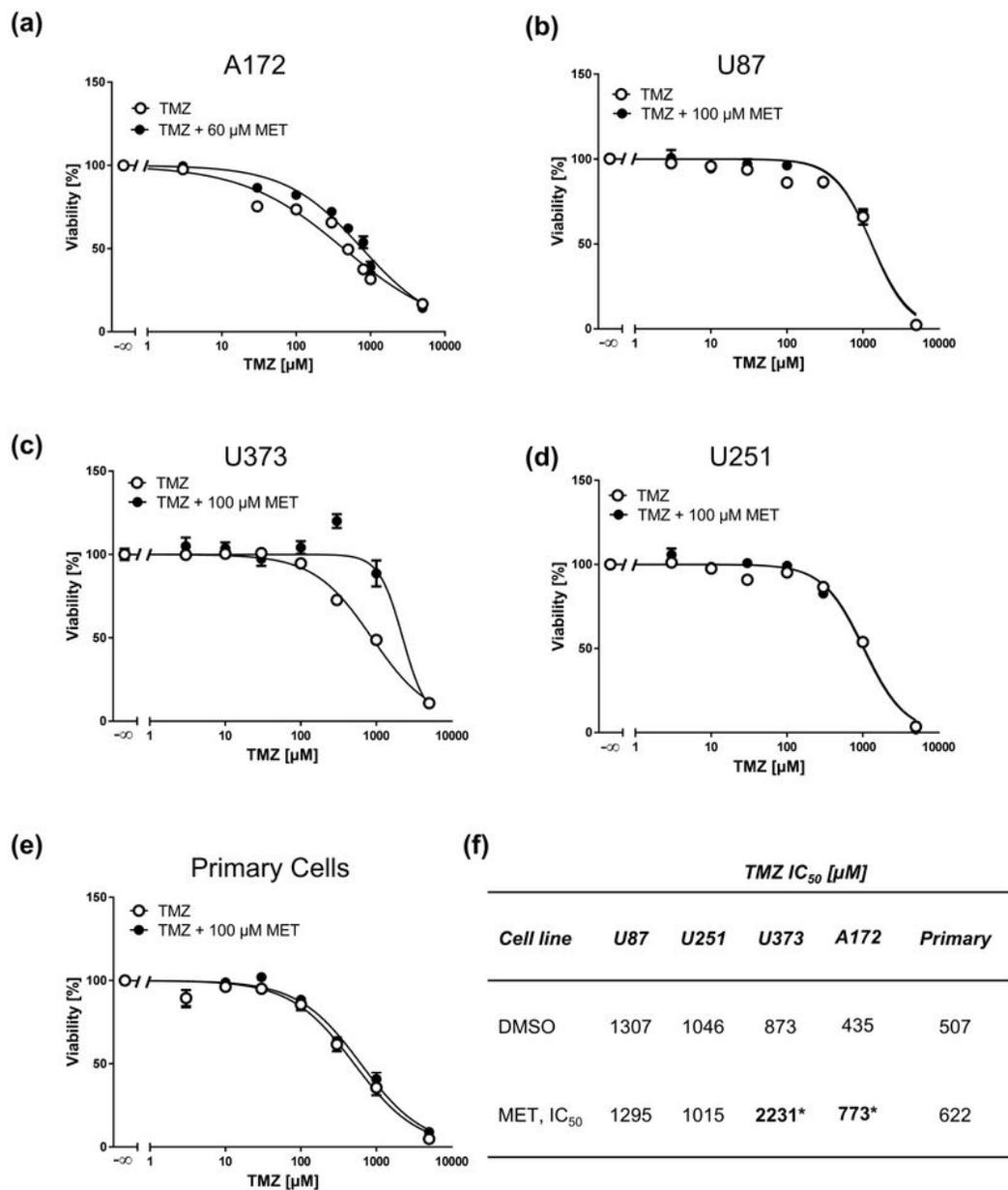
Figure 5

Sensitization of GBM cells by high dose MET towards DOXO is cell line-dependent. MTT assays of four GBM cell lines and primary cells treated with increasing concentrations of MET for 72 h as indicated. 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 IC50 concentration of MET for 1 h. (e) IC50 values derived from MTT assays of DOXO treated GBM cells with or without an IC50 concentration of MET. Values are displayed as mean +/- SEM (n = 3); \*, 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)).



**Figure 6**

MET (10  $\mu$ M) does not sensitize GBM cell lines and primary cells towards 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  $\mu$ M MET for 1 h. Values are displayed as mean  $\pm$  SEM (n = 3-4). (f)  $IC_{50}$  values of TMZ (vehicle) and TMZ + MET treated GBM cells derived from MTT assays; \*, p < 0.05.



**Figure 7**

IC<sub>50</sub> concentrations of MET do not sensitize GBM cell lines and primary cells towards 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 alone and in combination with an IC<sub>50</sub> concentration of MET for 72 h as indicated. Values are displayed as mean +/- SEM (n = 3-4). (b) IC<sub>50</sub> values of TMZ (vehicle) and TMZ + MET treated GBM cells derived from MTT assays; \*, p < 0.05.