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Safety and Tolerance of D,L-Methadone in Combination with Chemotherapy in Patients with Glioma

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Abstract. *Background/Aim: D,L-Methadone increases sensitivity toward chemotherapy of different tumor cell populations. We evaluated the safety and tolerance of additional use of D,L-methadone in patients with glioma in combination with chemotherapy. Patients and Methods: The dosage, duration of therapy and side-effects related to D,L-methadone were recorded in 27 patients. Toxicity was assessed accordingly to the Common Toxicity Criteria (CTC) of the National Cancer Institute. Progression-free survival at 6 months (PFS-6) was assessed. Results: A total of 13 patients reported grade 1-3 nausea at the beginning of the D,L-methadone therapy. Four patients reported persistent side-effects of nausea (CTC Grade 2, n=1) and obstipation (CTC grade 2-3, n=3). PFS-6 of patients with glioblastoma was 80% in those with non-methylated O^6-methylguanine-DNA methyltransferase (MGMT) (n=5) and 100% in those with MGMT methylation (n=7). Conclusion: D,L-methadone can be safely combined with standard glioma chemotherapy without increasing the risk of toxicity or vegetative symptoms such as tachycardia, sweating and restlessness. PFS-6 in patients with primary glioblastoma treated this way seems to be at least comparable to that of historic controls.*

Glioblastoma multiforme (GBM) is the most frequent and malignant type of human brain tumor, which quickly recurs despite standard therapy (1). One possible way to overcome chemoresistance and tumor progression might be reached with the use of additional agents sensitizing tumor cells to applied therapies.

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Recently it has been shown that the opioid D,L-methadone increases sensitivity toward chemotherapy of different tumor cell populations (2, 3). Opioids are substances that act on opioid receptors. Opioid receptor activation initiates a cascade of events resulting in a diversity of biological effects such as analgesia and sedation but also has effects on cell survival and proliferation (4-7). Opioid receptor stimulation can activate inhibitory G_i-proteins, which in turn block adenylyl cyclase activity, reducing cyclic adenosine monophosphate (cAMP) (Figure 1) (8). Simultaneous administration of the μ-opioid receptor agonist D,L-methadone and doxorubicin led to an increased antitumor effect *via* reducing second messenger cAMP (2, 3). Down-regulation of cAMP sensitizes tumor cells for anticancer treatment and leads to increased rates of apoptosis of human glioma cells and glioma stem cells in cell culture (2). This effect is most pronounced with D,L-methadone since D-methadone stabilizes the opioid receptors and thus facilitates more binding of L-methadone. D,L-Methadone also down-regulates expression of anti-apoptotic molecules such as B-cell lymphoma-extra large (BCL-xL) or X-linked inhibitor of apoptosis protein (XIAP) (2, 3, 7). The effect of D,L-methadone is also dependent on the opioid receptor density on the tumor cell surface and the agent's dosage (2, 3). As a proof-of-principle, the therapeutic effect is reversible by either blocking opioid receptors with naloxone administration, or by inhibiting Gi-proteins with pertussis toxin, leading to a subsequent increase in cAMP or by up-regulation of cAMP through inhibition of cAMP phosphodiesterases using 3-isobutyl-1-methylxanthine (2, 3). In a subcutaneous glioma model, D,L-methadone inhibited tumor growth significantly *in vivo* (2).

D,L-Methadone has played a role in palliative care for decades and is in use for pain treatment in patients with cancer (9). Previous studies showed that low-dose D,L-methadone (dosage of 15 mg daily) is well tolerated and results in pain control without dose escalation or opioid-induced hyperalgesia (10, 11). Care has to be taken regarding possible cardiotoxic events with higher doses and prolonged administration (12). Since the supposed anticancer effect of D,L-methadone is

propagated among clinical oncologists, the use of D,L-methadone in place of other commonly used analgesics is increasing. However, there are no reliable data supporting the antitumor activity of D,L-methadone application in patients with cancer in prospective clinical studies.

This study was designed to provide data for safety and tolerability combining D,L-methadone and chemotherapy in patients with glioma who received D,L-methadone from their palliative caregivers or clinical oncologists. D,L-Methadone was prescribed as part of an individual therapeutic trial and not as an analgesic or as a substitute for substance abuse. The regimen has been widely discussed in patient social media (online forum, *etc.*) and gained popularity among neuro-oncological patients in Germany.

Patients and Methods

Study design and treatment. We performed a retrospective study including 27 patients with glioma diagnosis grade II-IV according to the WHO classification (13), who received additional D,L-methadone independently from their neuro-oncological treatment. D,L-Methadone was used as part of an individual therapeutic regimen that was suggested by a clinical oncologist or was requested by the patient.

All patients used 1% D,L-methadone hydrochloride solution (1 g D,L-methadone hydrochloride, 0.06 g sorbic acid, 0.08 g citric acid and 100 ml purified water). The ascending dosing protocol included a starting dose of 2.5 mg *bid* and step-wise increase of methadone dosage every 3-5 days by 2.5 mg daily up to a maximum of 20-35 mg per day in two single doses. Patients increased dosage individually according to tolerance within the first 4 weeks.

Histological diagnosis was confirmed after biopsy or resection in all patients.

Patient information collected included demographic data, date of initial diagnosis, extent of surgical resection, details of all adjuvant therapies, reason for D,L-methadone prescription, beginning of D,L-methadone intake and time to tumor recurrence. Specific patient data were recorded concerning side-effects of D,L-methadone, including nausea, vomiting, anxiety, fatigue or other symptoms (obstipation, drowsiness, pruritus, sweating and hematotoxicity) and side-effects not further specified (*e.g.* elevated heart rate). Patients who received enzyme-inducing antiepileptic drugs (*e.g.* phenytoine, carbamazepine or valproic acid) were switched to non enzyme-inducing antiepileptic drugs (*e.g.* levetiracetame, lamotrigine, or lacosamide) before starting cytostatic therapy (14).

Details of individual treatment were derived from hospital charts, medical reports and an individual questionnaire. These data were assessed using common toxicity criteria (CTC) of the National Cancer Institute (NCI) version 4.03 of 2010 (15). The local Ethics Committee approved the trial (EA2/040/16).

Patient evaluation. Patients and caregivers completed a standardized questionnaire including biometrical data, clinical status, and details of antitumor therapy (dosage, continuity of certain chemotherapy) and side-effects. The questionnaire is included in Table I. Adjuvant therapy dosage of irradiation and chemotherapy as well as toxicity in accordance to the CTC criteria were recorded. A radiologist and clinical oncologist/neuro-oncologist reviewed the magnetic resonance imaging data in all patients. Time to progression was estimated in regards to Response Assessment in Neuro-Oncology criteria clinically and on magnetic resonance imaging (16). Progression-free survival (PFS) and progression-free survival at 6 months (PFS-6) were selected for evaluating therapeutic response (17, 18). Survival data for the intervention group was compared to a historic patient population (historic control group) treated at our Institution between 2012-2015. Patient characteristics of both groups are given in Table II.

D,L-Methadone dosage, ascending dosing protocol and additional drugs to counteract side-effects during initial intake and in the maintenance phase of the therapy were recorded. Tolerability and safety was assessed in regards of CTC criteria including nausea, vomiting and hematotoxicity. Furthermore, we recorded other symptoms such as fatigue, anxiety, drowsiness, pruritus, sweating or obstipation.

Statistical analysis. Statistical calculation was performed using Prism, version 5.0c (GraphPad Software, San Diego, CA, USA) and Excel (Microsoft, Redmond, Washington, USA). Results were analyzed using the intergroup comparison with Cox regression analysis. The level of acceptable significance was set at $p<0.05$.

Results

Between November 2014 and September 2015, 27 patients were included in this retrospective analysis. All patients received D,L-methadone from palliative caregivers or clinical oncologists. Of these, 13 patients were treated at initial diagnosis of GBM, seven at first recurrence of GBM, six patients at recurrence of anaplastic astrocytoma or anaplastic oligoastrocytoma, and one patient with a newly diagnosed low-grade optic glioma. Detailed patient characteristics including O^6-methylguanine-DNA methyltransferase (*MGMT*) status are given in Table III.

In 12 patients with GBM, D,L-methadone was combined with the first-line chemotherapy (Stupp regimen: post irradiation adjuvant cyclic temozolomide on 5/28 days, 150-200 mg/m^2). Of these, two patients started D,L-methadone parallel with radiochemotherapy. Seven out of 12 patients with first diagnosis of GBM had hypermethylation of the *MGMT* promotor, the remaining five patients had no hypermethylation of the *MGMT* promotor. Six patients with GBM started D,L-methadone at first recurrence, one patient at second recurrence. At the stage of first, second or third recurrence of a malignant glioma (n=13), D,L-methadone was combined with different anticancer therapies: lomustine in one, procarbacin plus lomustine in two, bevacizumab in one, cyclic temozolomide on 5/28 days in five, temozolomide one week on/one week off in two, bevacizumab plus lomustine in one or metronomic temozolomide in one.

Dosing of D,L-methadone. Patients usually started with stepwise increase of D,L-methadone dosage over a period of 2-3 weeks. Starting doses of 5 drops *bid* (5 mg daily dose) were increased with 1-5 drops every 5 days (5 drops is equivalent to 2.5 mg) according to the tolerance of the patients. All patients reported

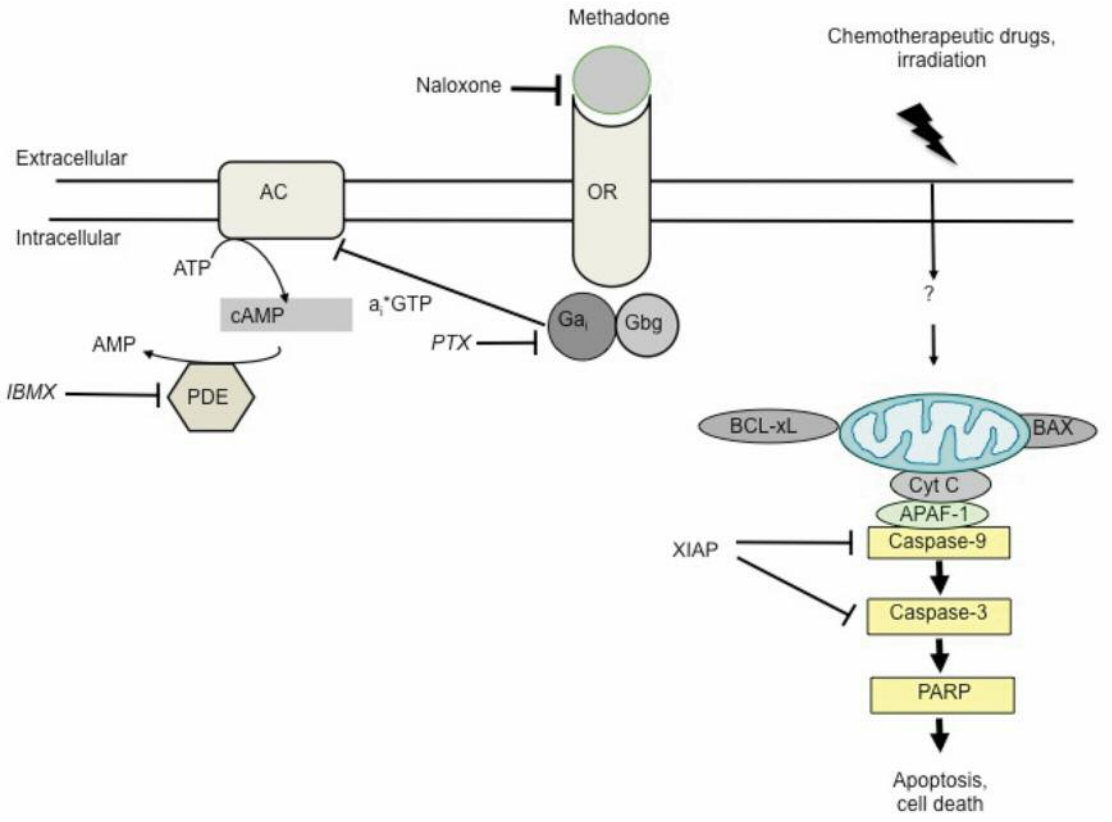

Figure 1. *D,L-Methadone and signaling pathways. D,L-Methadone (Methadone) sensitizes tumor cells to anticancer therapy and induces apoptosis via activation of opioid receptor signaling pathways. Triggering opioid receptors (OR) using D,Lmethadone leads to down-regulation of cyclic adenosine monophosphate (cAMP) which further down-regulates the anti-apoptotic proteins X-linked inhibitor of apoptosis protein (XIAP) and B-cell lymphoma-extra large (BCL-XL) and activates caspase-9 and caspase-3, cleaving poly (ADP-ribose) polymerase (PARP), inducing apoptosis. Blocking opioid receptor signaling pathways using naloxone, 3-isobutyl-1-methylxanthine (IBMX) or pertussis toxin (PTX) inhibits D,L-methadone, sensitizing tumor cells to anticancer treatment and blocks D,L-methadone-related apoptosis induction. AC: Adenylylcyclase, APAF-1: apoptosis protease activating factor-1, AMP: adenosine monophosphate, ATP: adenosine triphosphate, BAX: BCL-2-associated X protein, cyt C: cytochrome c, G: G proteins (Gα, Gβ/Gγ subunits), OR: opioid receptor, PDE : phosphodiesterase.*

consistent intake of D,L-methadone for a period of at least 3-12 months. Final dosage of D,L-methadone ranged between 15-35 mg per day. Serum plasma level of D,L-methadone was measured in one patient. Under 30 mg of D,L-methadone per day, a basal plasma concentration of 182 ng/ml was measured, at 1 to 2 hours after D,L-methadone intake plasma concentration was 206 ng/ml and 198 ng/ml. The therapeutic plasma concentration of D,L-methadone ranges from 0.05 to 0.5 μg/ml; toxic level is reached with 1 μg/ml (19).

Safety. Within the period of increasing dosage from 5-25 mg per day over 2 to 4 weeks, 13/27 patients suffered from side-effects (Table IV). During that time, eight patients experienced CTC grade 1-3 nausea (Figure 2a). One patient reported fatigue, two patients reported anxiety and drowsiness, obstipation (n=1), sweating and pruritus (n=1) within the first 4 weeks of therapy. These symptoms resolved fully in two-thirds of patients after one month of therapy. Remaining symptoms in a total of four patients beyond 4 weeks were obstipation (n=3) and nausea (n=1).

During the first 4 weeks of D,L-methadone intake, prophylactic antiemetic drugs were prescribed and used in 59% of the cases. Antiemetic substances varied and prescription depended on the individual experience of the participating institutions. In 10/27 patients a combination of haloperidol and D,L-methadone was used, which led to symptom relief in the majority of the cases (6/10 patients); in the other four out of these 10 patients, side-effects from haloperidol out weighed those of D,L-methadone. Our survey revealed equivalent efficacy for alternative, antiemetic drugs alone or in combination such as ondansetron in four, dimenhydrinate in one and metoclopramide or palonosetron in one patient. Detailed information about duration, efficacy and tolerability of antiemetic drugs in combination with D,L-methadone are given in Table IV.

Table I. *Questionnaire for tolerability to D,L-methadone in patients with brain tumors.*

1. Last name, surname:
   a. Phone:
   b. E_mail:
   c. Date of birth:
   d. Age:
   e. Karnofsky Performance Score:
   f. Height:
   g. Weight:
   h. Body surface
2. Date of first operation
   a. Resection or biopsy?
   b. Multifocal tumor growth? More than 1 tumor?
   c. Tumor location? Which lobe?
   d. Complete resection?
3. What is the tumor grade and histological diagnosis?
   a. What is the MGMT methylation status?
   b. What ist the 1p19q status?
   c. Is ATRX expression lost in the tumor tissue?
4. What therapy was done after the operation?
   a. Did any fatigue, nausea or blood count abnormalities occur under first line therapy? If yes, please specify
5. Did you experience tumor recurrence?
   a. Second surgery for recurrence?
   b. What was second line treatment? Radiation? Study treatment? Chemotherapy? Please specifiy.
6. Did the tumor re-grow/progress despite second line therapy? What was the therapy for 2nd progression/recurrence?
7. Any study treatment?
   a. Were you treated using NovoCure/optune, Bevacizumab/Avastin or DC-Vax (dendritic cell) therapy?
8. When did you start taking D,L-methadone (date)?
   a. Did you take D,L-methadone in first line therapy or recurrence?
   b. Did the tumor recur under D,L-methadone therapy?
   c. What was the dosage of D,L-methadone?
   d. How long was the run-in phase?
   e. Did you experience nausea, vomiting, anxiety, confusion, fatigue or other symptoms (obstipation, drowsiness) in the run-in phase that could be related to D,L-methadone?
   f. Did you take additional medication to counteract side effects of D,L-methadone?
   g. Did nausea, vomiting, anxiety, confusion, fatigue or other symptoms (obstipation, drowsiness) occur under continuous therapy with D,L-methadone?
   h. If yes, please specify?
   i. Did you pause or stop taking D,L-methadone or was the dose adjusted?

Beyond the first 4 weeks of D,L-methadone intake, 4/27 patients reported persistent side-effects of mild to moderate nausea (CTC grade 2, n=1) and obstipation (CTC grade 2-3, n=3). All other patients reported CTC grade 0 or 1 side-effects (Figure 2b). One patient ended therapy due to persistent obstipation (Table IV). We did not find evidence that D,L-methadone intake increased pre-existing toxicity due to chemotherapy (data not shown). No further side-effects, such as edema and urinary retention, or any signs of cardiac toxicity were reported. No patients were hospitalized due to side-effects, and no deaths occurred within 3 months of methadone therapy.

Efficacy. A total of 12 patients with primary diagnosis of GBM received D,L-methadone with a daily dose 20-35 mg in parallel with the Stupp regimen (20). They had a PFS-6 of 91%. Among patients who started D,L-methadone therapy with recurrent glioblastoma, PFS-6 was 47%; the daily D,L-methadone dose ranged between 15 and 35 mg (n=6). Administering D,L-methadone as solitary rescue therapy at disease progression with estimated life expectancy of less than 12 weeks, disease progression was observed in 100% of cases (data not shown). Thus, in this cohort we recorded a minimum of stable disease at 6 months in almost half of the patients, even in those with recurrent disease when combined with chemotherapy. Individual outcome parameters for each patient are given in Table III.

In comparison to our historical control group and patient collectives from the literature who received the same therapeutic regimen, combination of D,L-methadone seems to have had no adverse effect concerning PFS-6 regardless of the stage of disease (18, 21, 22). In primary GBM with hypermethylation of the *MGMT* promoter, gross total tumor resection followed by Stupp treatment resulted in all patients reaching PFS-6 (n=7);

Table II. *Patient characteristics according to cohort.*

Characteristic	Intervention group (n=12) n (%)	Historic control group (n=38) n (%)[a]
Diagnosis	pGBM	pGBM
Male	8 (67)	22 (58)
Female	4 (33)	16 (42)
Age		
<50 years	4 (33)	9 (24)
>50 years	8 (67)	29 (76)
Extent of surgery		
Biopsy	1 (8)	0 (0)
Partial resection	2 (17)	0 (0)
Gross total resection	9 (75)	38 (100)
Stupp treatment completed	12 (100)	38 (100)
MGMT +	7 (58)	38 (100)
MGMT –	5 (42)	

MGMT: O^6-Methylguanine-DNA methyltransferase, MGMT +: hypermethylation of the *MGMT* promotor region, MGMT –: no hypermethylation of the *MGMT* promotor region, pGBM: primary glioblastoma multiforme. [a]Historic control group of patients with GBM treated at Charité Berlin between 2012-2015.

in primary GBM without hypermethylation of the *MGMT* promoter, 80% of patients reached PFS-6 (n=6, Table V).

Subgroup analysis of patients meeting the inclusion criteria of primary diagnosis of GBM, hypermethylation of the *MGMT* promotor, gross total tumor resection and initiation of Stupp therapy in combination with (intervention group with *MGMT* hypermethylation, n=7) and without (historical control group, n=37) D,L-methadone was performed. PFS-6 of the intervention group was found to be superior to that of the control group from our clinic (100% *vs*. 79%). Comparison of the PFS between the intervention group and control group revealed a relative risk reduction for tumor recurrence for the intervention group with *MGMT* hypermethylation, with a hazard ratio of 0.6420, which was, however, not statistically significant (Cox regression analysis, confidence interval=0.1781 to 2.315, p=0.3435; Figure 2c). Comparing PFS, we found a relative risk reduction for tumor recurrence for the intervention group of patients without hypermethylation of the *MGMT* promotor compared to the control group, with a hazard ratio of 0.6315, which again was not statistically significant (Cox regression analysis, confidence interval=0.1188 to 3.358, p=0.6456; Figure 2c). Detailed patient characteristics are given in Table II. Median overall survival was not reached in the intervention group and is therefore not reported.

Discussion

In this study, we showed for the first time that simultaneous intake of D,L-methadone and chemotherapeutics such as

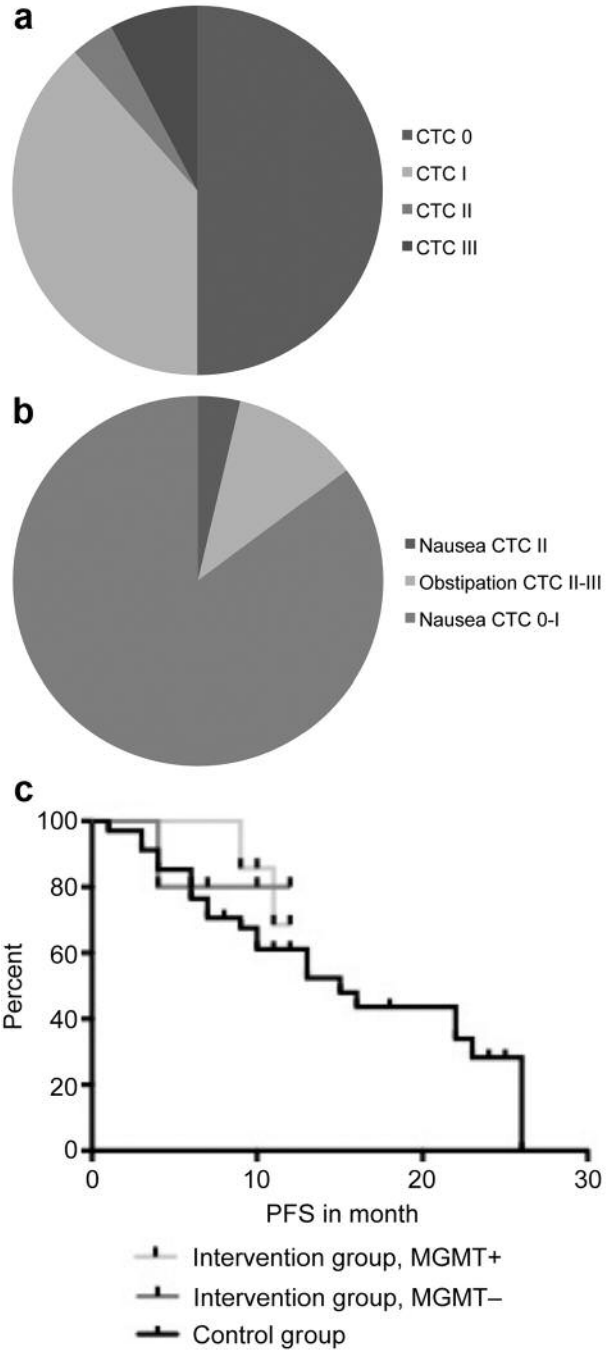

Figure 2. *Side-effects of D,L-methadone and outcome in patients with glioma. D,LMethadone-induced side-effects according to Common Toxicity Criteria v4.03 of the National Cancer Institute (CTC) within the first 4 weeks of therapy (a) and beyond the first 4 weeks of therapy (b). c: Progression-free survival (PFS) analysis of the intervention group with hypermethylation of the O-6-methylguanine-DNA methyltransferase promotor region (MGMT+, n=7) and control group (n=38) revealed a hazard ratio of 0.6420, confidence interval = 0.1781 to 2.315, p=0.3435. Analysis of PFS for the intervention group without hypermethylation of the MGMT promotor region (MGMT–, n=5) and historic control group (n=38) revealed a hazard ratio of 0.6315, confidence interval=0.1188 to 3.358, p=0.6456.*

Table III. *Patient characteristics*.

Pat ID	dx	*MGMT* status	Initial surgical therapy	KPS at D,L-methadone start	Recurrence under D,L-methadone	PFS (months)	Duration of D,L-methadone therapy (months)	Initial chemotherapy
Primary glioblastoma multiforme								
1	pGBM	+	GTR	100	No	11	11	cTMZ
5	pGBM	+	GTR	90	No	SD 12 months after dx	6	cTMZ
6	pGBM	+	GTR	90	No	SD 12 months after dx	6	cTMZ
13	pGBM	+	GTR	100	No	SD 12 months after dx	12	cTMZ
17	pGBM	+	GTR	100	No	SD 9 months after dx	10	cTMZ
22	pGBM	+	GTR	100	No	SD 12 months after dx	18	cTMZ
27	pGBM	+	GTR	90	No	SD 10 months after dx	6	cTMZ
3	pGBM	−	Bx	70	Yes	4	6	cTMZ
10	pGBM	−	PR	100	No	SD 10 months after dx	11	cTMZ
11	pGBM	−	Bx	90	No	SD 12 months after dx	11	cTMZ
23	pGBM	−	GTR	100	No	SD 6 months after dx	2	cTMZ
25	pGBM	−	GTR	80	No	SD 7 months after dx	6	cTMZ
21	pGBM	n.k.	n.k.	n.k.	Yes	n.k.	n.k.	n.k.
Recurrent glioblastoma multiforme								
20	1st rGBM	+	GTR	n.k.	Yes	SD 15 months after dx of 1st rGBM	11	cTMZ
15	1st rGBM	−	GTR	90	No	2	9	TMZ 1 week on/ 1 week off
18	1st rGBM	−	GTR	100	Yes	4	10	CCNU
12	1st rGBM	n.k.	GTR	100	No	SD 11 months after dx of 1st rGBM	11	cTMZ
24	1st rGBM	n.k.	GTR	n.k.	No	SD 16 months after dx	11	cTMZ
16	1st rGBM	n.k.	GTR	80	No	SD 8 months after dx of 1st rGBM	8	TMZ 1 week on/ 1 week off
26	2nd rGBM	n.k.	GTR	70	Yes	3	3	mTMZ
Anaplastic and diffuse gliomas								
19	pAOA	n.k.	GTR	100	No	SD 22 months after dx	10	PC
4	1st rAA	+	GTR	80	Yes	10	10	BEV+CCNU
8	1st rAA	n.k.	PR	90	No	SD 9 months after dx of 1st rAOA	3	PC
14	1st rAOA	+	GTR	100	No	SD 13 months after dx of 1st rAOA	2	cTMZ
2	2nd rAOA	−	PR	50	Yes	12	4	BEV
7	2nd rAOA	n.k	GTR	80	Yes	10	7	PC
9	pdiffA	n.k.	Bx	90	Yes	9	3	cTMZ

1st rAA: First recurrence of anaplastic astrocytoma, 1st rAOA: first recurrence of anaplastic oligo-astrocytoma, 1st rGBM: first recurrence of glioblastoma multiforme, 2nd rAOA: second recurrence of anaplastic oligo-astrocytoma, 2nd rGBM: second recurrence of glioblastoma multiforme, AA: anaplastic astrocytoma, AOA: anaplastic oligoastrocytoma, BEV: bevacizumab, Bx: biopsy, CCNU: lomustin, cTMZ: cyclic temozolomide, dx: diagnosis, GBM: glioblastoma multiforme, GTR: gross total resection, KPS: Karnofsky performance score, *MGMT*: O^6-methylguanine-DNA methyltransferase, mTMZ: metronomic temozolomide, n.k.: not known, Pat ID: patient identification number, pAOA: primary anaplastic oligoastrocytoma, pdiffA: primary diffuse astrocytoma, PC: procarbazine plus lomustine, pGBM: primary glioblastoma multiforme, PFS: progression-free survival, PR: partial resection, rAOA: recurrent anaplastic oligoastrocytoma, rAA: recurrent anaplastic astrocytoma, SD: stable disease, TMZ: temozolomide, +: hypermethylation of MGMT promotor region, −: no hypermethylation of *MGMT* promotor region.

temozolomide is well tolerated by the majority of patients with gliomadespite at an adjusting phase of 2 to 4 initial weeks. Our investigation of the impact of D,L-methadone intake on outcome shows that D,L-methadone had no detrimental effect in our small retrospective cohort of patients.

Little is known about tolerance and side-effects associated with combining temozolomide and other chemotherapeutics in glioma therapy with D,L-methadone. In this study, D,L-methadone was combined with standard dose radiochemotherapy or chemotherapy alone, low-dose chemotherapy or antiangiogenic substances such as bevacizumab prescribed by palliative caregivers and clinical oncologists. We show that D,L-methadone does not increase pre-existing hematotoxicity. Furthermore, D,L-methadone intake was well tolerated by the majority of patients after the first 4 weeks of dose escalation. We found a good tolerance of and high compliance with D,L-methadone therapy in

Table IV. *D,L-Methadone tolerability in patients in individual therapeutic trial*.

Pat ID	Dosage *bid* in drops	Nausea according to CTC weeks 1-4	Vegetative symptoms weeks 1-4	Aagent added	Side-effects beyond week 4
2	n.k.	n.a.	n.a.	None	n.a.
3	25	0	None	Haloperidol	No
4	20	0	Fatigue	None	No
5	n.k.	0	None	None	No
8	25	0	None	None	No
10	20	0	None	None	Obstipation
11	20	0	None	Haloperidol	No
12	20	0	None	Haloperidol	No
13	30	0	None	None	No
15	30-35	0	None	Ondansetron	No
16	35	0	None	Ondansetron	No
18	35	0	None	None	No
22	25	0	None	None	No
27	20	0	None	None	No
1	24	1	None	Haloperidol ondansetron	No
14	n.k.	1	None	Haloperidol	No
17	35	1	Anxiety, drowsiness	Haloperidol	No
19	20	1	Sweating, pruritus	Dimenhydrinate, polyethylenglycol and globuli	Obstipation
20	15	1	Nausea	Haloperidol	No
21	30	1	Nausea	None	No
23	20	1	Nausea	Haloperidol	No
24	25	1	Nausea	Haloperidol	No
25	25	1	Nausea	Haloperidol, palonosetron	No
26	20	1	None	None.	No
7	20	2	None	Pantoprazole	No
6	20	3	Obstipation	Ondansetron	Obstipation
9	16	3	Anxiety, drowsiness	Ondansetron followed by metoclopramide	Nausea CTC grade 2

bid: Twice a day, CTC: Common Toxicity Criteria of the National Cancer Institute, n.a.: not assessable, n.k.: not known, Pat ID: patient identification number.

Table V. *Outcome in patients with glioblastoma multiforme (GBM)*.

Diagnosis	n	PFS-6 intervention group (%)	PFS-6 historic control groups (%) (Ref)
Primary GBM	13	91.6	53.9 (26)
MGMT +	7	100	79[a]
MGMT –	5	80	40 (29)
1st Recurrence of GBM	6	47	20-50 (22, 29)

MGMT+: Hypermethylation of the O^6-methylguanine-DNA methyltransferase (*MGMT*) promotor region, *MGMT*–: no hypermethylation of the *MGMT* promotor region, PFS-6: progression-free survival at 6 months after diagnosis. [a]Historic control group of patients with GBM treated at Charité Berlin between 2012-2015.

combination with chemotherapy, which might indicate its use in patients with glioma with severe headache or other pain syndromes (23).

The risk of hypoglycemia due to D,L-methadone intake is reportedly increased compared to other opioids (24). With an average intake of 15-30 mg/day in our cohort, dosage was below the critical level of 40 mg/day associated with developing hypoglycemia and none of our patients showed clinical signs or proven hypoglycemia. Further side-effects such as edema or urinary retention were not reported. There are conflicting data regarding D,L-methadone dose- and treatment duration-dependency of cardiac toxicity (12). A recent review reported ECG abnormalities in patients receiving a daily dosage of 80-125 mg (25). This far exceeds the daily dose used in our study, which might explain why neither signs of cardiac toxicity nor clinical signs of abnormal tachycardia in the patient cohort were reported. Moreover, none of the patients were hospitalized due to side-

effects and no deaths occurred within 3 months of methadone therapy. Regular ECG monitoring is advised in the treatment phase to detect prolongation of QT time.

The main reported side-effect caused by D,L-methadone was nausea. We observed that prophylactic haloperidol helped control side-effects in the majority of cases. But, in some cases, side-effects from haloperidol itself outweighed those of D,L-methadone. With this study, we confirm that ondansetron or aprepitant effectively prevented side-effects. Although not a first-line antiemetic for chemotherapy-associated nausea, metoclopramide was also effective. Further escalation of antiemetic regimes with steroids or other substances might contribute to an improved control of these symptoms.

In the context that D,L-methadone was prescribed in an individual therapeutic trial to improve tumor control, an outcome analysis of subgroups concerning PFS-6 and PFS was carried out. PFS-6 of the intervention group appeared to be superior compared to the control group from our institution (100% *vs*. 80%) and compared to the literature (26, 27). However the potential benefit of the administration of D,L-methadone in combination with standard first-line therapy in the intervention group is apparent in the group with *MGMT* hypermethylation and even in those without. Progression-free survival of the intervention and control groups at first-line therapy was comparable.

Due to the small number of patients and retrospective study design, a final conclusion regarding improved outcome due to D,L-methadone administration cannot be made. In recurrent disease of malignant gliomas, heterogeneity of the patient population in terms of histological diagnosis, molecular profile, pre-existing therapies and stage of disease is significant. Moreover, the rate of metabolism of opioids differs between individually, which may lead to a different treatment effect in 'slow metabolizers' from that in 'fast metabolizers' due to different drug levels. Further drug interactions between D,L-methadone and chemotherapy metabolism *via* cytochrome P450 3A4 (CYP3A4) and others need to be ruled out. The significant advantage of D,L-methadone is that is has no active metabolites which could potentially interfere with kidney and liver function (Inturrisi *et al*. 1987). Since temozolomide is hydrolyzed to its pharmacologically active metabolite by physiological pH in a non enzyme-dependent way, the cytochrome pathway only plays a minor role in its metabolism (28, 29).

Despite the promising results from laboratory data and the safe and well-tolerated application of D,L-methadone in glioma patients found in this study, reliable clinical data are lacking, providing evidence that D,L-methadone has substantial antitumor effects in patients with glioma. At present D,L-methadone prescription should only be considered in patients with glioma with substantial indications. When considering D,L-methadone use as part of an individual anticancer therapy, the current lack of clinical data needs to be explained openly to the patient and physicians. It is mandatory that therapeutic decisions must not depend on D,L-methadone usage. In order to anticipate further speculations and criticism concerning D,L-methadone prescription in patients with glioma and other tumor types, the antitumor effect of D,L-methadone needs to be investigated in a prospective randomized clinical trial with respect to metabolism and tolerability to the drug in combination with approved, standard chemotherapeutic agents.

Conflicts of Interest

The Authors declare no conflict of interest in regard to this study.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study. The local Ethics Committee approved the trial (EA2/040/16).

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