JNS

CLINICAL ARTICLE

J Neurosurg 136:1542–1550, 2022

Influence of dexamethasone on visible 5-ALA fluorescence and quantitative protoporphyrin IX accumulation measured by fluorescence lifetime imaging in glioblastomas: is pretreatment obligatory before fluorescence-guided surgery?

Lisa I. Wadiura, MD,[1,6] David Reichert, MSc,[2,3] Veronika Sperl, BSc,[1,6] Alexandra Lang, MSc,[1,6] Barbara Kiesel, MD,[1,6] Mikael Erkkilae, MSc,[2] Adelheid Wöhrer, MD, PhD,[4,6] Julia Furtner, MD, PhD,[5,6] Thomas Roetzer, MD,[4,6] Rainer Leitgeb, PhD,[2,3] Mario Mischkulnig, MD,[1,6] and Georg Widhalm, MD, PhD[1,6]

[1]Department of Neurosurgery; [2]Center for Medical Physics and Biomedical Engineering; [3]Christian Doppler Laboratory OPTRAMED; [4]Department of Neurology–Division for Neuropathology and Neurochemistry; [5]Department of Biomedical Imaging and Image-guided Therapy, Division of General and Pediatric Radiology; and [6]Comprehensive Cancer Center–Central Nervous System Tumors Unit, Medical University of Vienna, Austria

OBJECTIVE Fluorescence-guided surgery using 5-aminolevulinic acid (5-ALA) is nowadays widely applied for improved resection of glioblastomas (GBMs). Initially, pretreatment with dexamethasone was considered to be essential for optimal fluorescence effect. However, recent studies reported comparably high rates of visible fluorescence in GBMs despite absence of dexamethasone pretreatment. Recently, the authors proposed fluorescence lifetime imaging (FLIM) for the quantitative analysis of 5-ALA–induced protoporphyrin IX (PpIX) accumulation. The aim of this study was thus to investigate the influence of dexamethasone on visible fluorescence and quantitative PpIX accumulation.

METHODS The authors prospectively analyzed the presence of visible fluorescence during surgery in a cohort of patients with GBMs. In this study, patients received dexamethasone preoperatively only if clinically indicated. One representative tumor sample was collected from each GBM, and PpIX accumulation was analyzed ex vivo by FLIM. The visible fluorescence status and mean FLIM values were correlated with preoperative intake of dexamethasone.

RESULTS In total, two subgroups with (n = 27) and without (n = 20) pretreatment with dexamethasone were analyzed. All patients showed visible fluorescence independent from preoperative dexamethasone intake. Furthermore, the authors did not find a statistically significant difference in the mean FLIM values between patients with and without dexamethasone pretreatment (p = 0.097).

CONCLUSIONS In this first study to date, the authors found no significant influence of dexamethasone pretreatment on either visible 5-ALA fluorescence during GBM surgery or PpIX accumulation based on FLIM. According to these preliminary data, the authors recommend administering dexamethasone prior to fluorescence-guided surgery of GBMs only when clinically indicated.

https://thejns.org/doi/abs/10.3171/2021.6.JNS21940

KEYWORDS 5-ALA; 5-aminolevulinic acid; dexamethasone; visible fluorescence; fluorescence lifetime imaging; glioblastoma; oncology

Neurosurgical resection is the initial treatment of choice in patients suffering from glioblastomas (GBMs).[1,2] In surgery of GBMs, the extent of resection is a key factor for patient prognosis.[1,3–5] Therefore, the aim of neurosurgical tumor resection is maximal safe removal of GBM tissue whenever possible.[1,3–5] In 1998, the use of 5-aminolevulinic acid (5-ALA)–induced fluorescence was introduced to the neurosurgical field for improved intraoperative visualization of GBM tissue and the tumor margin.[6] After preoperative peroral administra-

ABBREVIATIONS 5-ALA = 5-aminolevulinic acid; CE = contrast enhancement; FLIM = fluorescence lifetime imaging; GBM = glioblastoma; PpIX = protoporphyrin IX.

SUBMITTED April 10, 2021. **ACCEPTED** June 7, 2021.
INCLUDE WHEN CITING Published online October 22, 2021; DOI: 10.3171/2021.6.JNS21940.

TABLE 1. Literature overview on studies of brain tumors with data on dexamethasone and 5-ALA fluorescence

Authors & Year	No. of Pts	Tumor Entity	Rate of Fluorescence	Type of Op	Pretreatment w/ Dexamethasone	Study Results Related to Dexamethasone	Fluorescence Detection
Stummer et al., 2000[25]	52	GBM	52/52 (100%)	Resection	In all patients*	†	Visible fluorescence
Panciani et al., 2012[24]	23	GBM	23/23 (100%)	Resection	In all patients*	†	Visible fluorescence
Kiesel et al., 2018[12]	77	GBM	77/77 (100%)	Resection	If clinically indicated	†	Visible fluorescence
Kiesel et al., 2018[42]	41	Lymphoma	32/41 (79%)	Biopsy	If clinically indicated	No influence of dexamethasone on fluorescence	Visible fluorescence
Lawrence et al., 2016[30]		GBM cell lines			72-hr treatment in therapeutic media	Dexamethasone reduced total amount of PpIX & increased intracellular accumulation	In vitro
Present study	47	GBM	47/47 (100%)	Resection	If clinically indicated	No significant influence of dexamethasone pretreatment on either visible or PpIX accumulation	FLIM, visible fluorescence

Pts = patients.

* No analysis of dexamethasone and fluorescence.

† Patients received 4 mg dexamethasone 3 times a day, at least 2 days before surgery.

tion of 5-ALA, this dye results in intratumoral accumulation of the actual fluorescing metabolite protoporphyrin IX (PpIX).[7] In a multicenter phase III trial, Stummer et al. demonstrated the superiority of 5-ALA fluorescence-guided surgeries with regard to the extent of resection and progression-free survival in GBMs as compared to conventional white-light resections.[2] Consequently, 5-ALA fluorescence-guided surgeries are nowadays standard for optimal resection of GBMs at many specialized centers worldwide.[8]

Generally, 5-ALA is able to visualize GBM tissue during surgery with strong fluorescence.[9,10] In the multicenter phase III trial by Stummer et al., pretreatment with dexamethasone (12 mg/day at least 2 days before surgery) was considered to be crucial for the efficacy of 5-ALA fluorescence-guided surgery.[2,7] The authors indicated that this assumption was based on the known corticosteroid-induced tightening effect of the blood-brain barrier and the likelihood of increased 5-ALA uptake.[2,7] In contrast, our group treats patients with dexamethasone before 5-ALA fluorescence-guided surgery of GBMs only if this is clinically indicated based on vasogenic edema or intracranial mass effect.[10–12] Despite the lack of pretreatment with dexamethasone, we observed comparably high rates of strong fluorescence in surgery of GBMs.[10,12] According to the current literature, it remains unclear whether the pretreatment of dexamethasone prior to 5-ALA fluorescence-guided surgery of GBMs is mandatory to achieve a sufficient intraoperative fluorescence effect. These data are of major importance for neurosurgeons to allow optimal preoperative management of patients scheduled for 5-ALA fluorescence-guided GBM surgery. A literature overview on studies of brain tumors with data on dexamethasone and 5-ALA fluorescence is provided in Table 1.

Routinely, the intraoperative assessment of 5-ALA fluorescence in surgery of GBMs is based on the surgeon's subjective opinion and thus requires a high level of experience.[13] In order to overcome this limitation, our group recently introduced fluorescence lifetime imaging (FLIM) for the quantitative and objective measurement of 5-ALA–induced PpIX accumulation in brain tumors even in the subvisual fluorescence range.[14,15] This novel technique is thus useful to determine differences in intratumoral 5-ALA–induced PpIX accumulation.[14–17]

The aim of this study was therefore to prospectively analyze the influence of dexamethasone on visible 5-ALA fluorescence and quantitative PpIX accumulation measured by FLIM in GBMs. To this end, we correlated the visible intraoperative 5-ALA fluorescence status as well as the average fluorescence lifetime measured by FLIM of representative GBM samples with the preoperative intake of dexamethasone.

Methods

We prospectively included patients who were treated between January 2019 and March 2021 with 5-ALA fluorescence-guided resection of a GBM at the Department of Neurosurgery, Medical University of Vienna, and who had postoperative ex vivo measurement of collected tumor samples with FLIM. In the present study, we used the following inclusion criteria: 1) adult patients (≥ 18 years); 2) suspicion of a GBM according to preoperative MRI, or recurrent GBM; 3) patients planned for fluorescence-guided resection using 5-ALA; 4) no contraindications for 5-ALA administration; and 5) available data on preoperative intake of dexamethasone. Patients had to be excluded from this study if postsurgical histological diagnosis was other than GBM WHO grade IV. This study was approved by the local ethics committee of the Medical University of Vienna and informed consent was given by the patients.

Preoperative Imaging

In the present study, diagnostic MRI including T1-weighted contrast-enhanced sequences was conducted in the preoperative course in all patients.[18,19] After admission to hospital, we additionally performed contrast-enhanced T1-weighted images, and dependent on the tumor localization, we also obtained functional MRI and/or diffusion tensor imaging for integration into the neuronavigation

system. With regard to the pattern of contrast enhancement (CE) on preoperative MRI, we only included patients with typical ringlike CE (newly diagnosed or recurrent GBMs). Furthermore, the tumor localization was categorized into 7 main localizations comprising temporal, parietal, insular, central, frontal, occipital, and thalamic regions. The pattern of CE and tumor localization on preoperative MRI was categorized by an experienced neuroradiologist (J.F.).

5-ALA Fluorescence-Guided GBM Resection

All tumor resections were assisted by image guidance with neuronavigation, white-light microscopy, and fluorescence-guided surgery. For this purpose, 5-ALA (20 mg/kg bodyweight) was perorally administered approximately 3 hours before anesthesia in all patients. During surgery, we applied a modified neurosurgical microscope (KINEVO; Carl Zeiss Surgical GmbH) for intraoperative assessment of visible 5-ALA fluorescence.[20] According to our strategy, the presence of visible fluorescence was analyzed in the contrast-enhancing tumor region based on neuronavigation. The maximum fluorescence level (strong, vague, or none) was documented by the operating neurosurgeon, and one representative tumor sample was safely collected from this area for ex vivo FLIM analysis.

Tumor Tissue Preparation

The collected tissue samples were ex vivo immersed in artificial CSF to ensure safe transportation to the imaging laboratory within 1 hour after tissue collection. The artificial CSF used contains isotonic contents (sodium chloride, potassium chloride, calcium chloride, magnesium chloride, glucose) to preserve the viability of the tissue samples through a physiological solution. After imaging data were obtained, tissue samples were transferred to the neuropathology department for histopathological evaluation.

FLIM System

All tumor samples were imaged ex vivo by using a custom-built frequency-domain FLIM system integrated into a surgical microscope (OPMI Visu 200; Carl Zeiss Meditec). A 405-nm laser (phoxX-405; Omicron Laserage) was modulated at 10 MHz for excitation. FLIM information was then encoded in the phase-shifted fluorescence emission and could be recovered by using a homodyne detection scheme. By raster-scanning the laser, lifetime maps over a field of view of approximately 6×6 mm^2 were acquired. A detailed description of the system and institutional imaging was previously published.[14] Measurements before September 2019 were performed with a dual-tap CMOS camera (pco.flim; PCO AG).[15] It is of note that the FLIM system is intrinsically independent of variations of excitation power, absorption/scattering in tissue, or the method of measurement.[21]

Postoperative Course and Histopathological Diagnosis

Routinely, all patients received protection from strong light sources for at least 24 hours after oral administration of 5-ALA to avoid potential skin phototoxicity. Histopathological tumor diagnosis was performed according to the valid WHO criteria at time of diagnosis.[22,23] Only patients with a confirmed histopathological diagnosis of a WHO grade IV GBM were included in this study. Following FLIM analysis, all tissue samples were histopathologically investigated for the presence of tumor cells.

Analysis of Clinical Data on Corticosteroid Intake

According to our strategy, dexamethasone treatment was only administered prior to surgery if patients presented with symptoms related to vasogenic edema, mass effect, and/or increased intracranial pressure. The clinical data on preoperative dexamethasone treatment were classified as "yes" or "no," and the total daily dose (in mg) was noted. Additionally, the duration of preoperative dexamethasone treatment was documented and classified as ≤ 7 days or > 7 days.

Statistical Analysis

Statistical analyses were performed using the statistical software SPSS (version 26.0; IBM Corp.). Descriptive investigations included patient age and sex, type of CE on MRI, tumor location, IDH1 mutation status, and mean FLIM values. In order to test for differences in FLIM values according to preoperative corticosteroid treatment, normal distribution was initially ruled out using the Shapiro-Wilk test, and analysis was thus performed using the nonparametric Mann-Whitney U-test. Given that only a single inferential statistical analysis was performed, no correction for multiple testing was required and statistical significance was thus assumed at p values below the typical threshold of $p = 0.05$.

Results

Altogether, 109 patients underwent resection of a GBM at our institution in the study period between January 2019 and March 2021. In total, we included 47 patients in our present study who had undergone 5-ALA fluorescence-guided tumor resection and in whom quantitative FLIM analyses were available. Therefore, we did not include sequential patients (62 of 109 patients) in our current study cohort. The main reasons for exclusion of patients from this study were as follows: 1) insufficient tumor tissue for subsequent FLIM analysis; 2) postoperative investigation with FLIM was not available; 3) known contraindications of 5-ALA administration; and 4) participation in other clinical studies.

In each patient, 5-ALA–induced PpIX accumulation was postoperatively investigated in representative samples of the tumor core by FLIM and correlated with preoperative intake of dexamethasone. In none of the patients were any significant side effects attributed to the 5-ALA administration observed in the perioperative course.

Patient Characteristics

The median age of our study cohort was 57 years (range 27–80 years) with a male/female ratio of 1.2:1. According to preoperative MRI, all tumors showed significant ringlike CE. Furthermore, 15 (32%) cases were localized in the parietal lobe, 14 (30%) cases in the frontal lobe, and 14

TABLE 2. Characteristics of 47 patients with GBMs

Characteristic	Value
No. of pts	47 (100)
Sex	
Male/female	1.2:1
Median age (range), yrs	57 (27–80)
Localization	
Parietal	15 (32)
Frontal	14 (30)
Temporal	14 (30)
Central	2 (4)
Occipital	1 (2)
Insular	1 (2)
Thalamic	0 (0)
Fluorescence status	
Visible fluorescence	47 (100)
No fluorescence	0 (0)
Fluorescence level	
Strong	43 (92)
Vague	4 (8)
None	0 (0)
Fluorescence homogeneity	
Inhomogeneous	47 (100)
Homogeneous	0 (0)
Dexamethasone preop	
Yes	27 (57)
No	20 (43)
Duration of administration	
≤7 days preop	23 (85)
>7 days preop	4 (15)

Values are presented as number (%) unless otherwise indicated.

(30%) cases in the temporal lobe. In the majority of GBMs, no IDH1 mutation was present (89%). Further details are provided in Tables 2 and 3.

5-ALA Fluorescence and FLIM Analysis in GBMs

During tumor resection, visible 5-ALA fluorescence was found in all of the 47 (100%) patients with GBMs, and representative tissue samples from the tumor core were collected for measurement of PpIX accumulation. According to ex vivo FLIM analysis, we found a mean value of 11.9 ± 2.2 nsec (range 4.2–14.7 nsec). All investigated tissue samples contained tumor cells according to histopathological analysis.

Preoperative Dexamethasone

In our study, 27 patients received preoperative dexamethasone due to neurological symptoms caused by vasogenic edema and intracranial mass effect. Of these, the total daily dose was 2 mg in 1 patient, 4 mg in 4 patients, 6 mg in 1 patient, 8 mg in 7 patients, 12 mg in 11 patients, 16 mg in 2 patients, and 24 mg in 1 patient. In the vast majority of patients (23 [85%] of 27 patients), the pretreatment with dexamethasone before 5-ALA fluorescence-guided tumor resection was ≤ 7 days. In contrast, 20 patients did not receive pretreatment with dexamethasone before GBM surgery. Further details are given in Table 2.

Correlation of Dexamethasone Intake and FLIM Analysis

According to FLIM analysis of 5-ALA–induced accumulation of representative tissue samples, we found a mean value of 12.3 ± 2.2 nsec in patients with dexamethasone pretreatment and 11.4 ± 2.2 nsec in patients without dexamethasone intake before surgery. Two representative cases of patients with and without pretreatment with dexamethasone before surgery of GBMs and PpIX fluorescence analysis measured by FLIM are provided in Figs. 1 and 2, respectively.

According to our data, we did not find a statistically significant difference in the mean FLIM values between patients with GBMs and without dexamethasone pretreatment ($p = 0.098$). Further details are provided in Fig. 3.

Discussion

In the past 20 years, PpIX-induced fluorescence after oral administration of 5-ALA has been successfully used as an intraoperative visualization tool in surgery of GBMs, resulting in an increased rate of gross-total resections.[2,8,12] According to the current literature the vast majority of GBMs demonstrate visible fluorescence during surgery.[8,12]

In 1998, at the time 5-ALA fluorescence-guided surgery of GBMs was introduced to the neurosurgical field, pretreatment with dexamethasone was considered to be mandatory to achieve an optimal intraoperative fluorescence effect.[6,24,25] However, even short-term therapy with corticosteroids is associated with multiple potential adverse effects such as venous thromboembolism, fracture, hyperglycemia, sleep disturbances, and an increased rate of sepsis.[26–29]

Subsequently, the authors of other studies did not administer dexamethasone routinely before fluorescence-guided surgery of GBMs and performed a pretreatment with corticosteroids before 5-ALA administration in patients only if clinically indicated.[11,12] Despite the absence of dexamethasone pretreatment, similarly high rates of visible fluorescence during surgery of GBMs were reported.[11,12] Nevertheless, pretreatment with dexamethasone might result in alterations in 5-ALA–induced PpIX accumulation not visible with the naked eye. Indeed, one in vitro study reported an increased intracellular retention of PpIX in GBM cell lines pretreated with dexamethasone.[30] In order to precisely detect 5-ALA–induced PpIX accumulation, recent studies focused on the analysis of quantitative fluorescence measured by novel techniques such as handheld spectroscopic probes or FLIM.[16,31–34] These techniques allow detection of even slight differences in fluorescence that escape notice during fluorescence-guided surgery.

So far, no systematic and prospective data are available in the current literature analyzing the influence of dexamethasone on the intraoperative 5-ALA fluorescence measured by quantitative techniques only in patients with GBMs (Table 1). These data would be of major importance

TABLE 3. Study cohort of 47 patients with GBMs

Case No.	Age (yrs)	Sex	Localization	Side	Recurrent Tumor	IDH1	Intraop 5-ALA Fluorescence	Level of Fluorescence	FLIM PpIX Mean Value (nsec)	SD	Dexamethasone Preop
1	72	M	Frontal	Rt	No	Wildtype	Yes	Strong	6.8	0.9	Yes
2	50	M	Temporal	Lt	No	Wildtype	Yes	Strong	13.1	0.6	Yes
3	73	F	Occipital	Rt	No	Wildtype	Yes	Strong	13.9	1.0	Yes
4	56	F	Frontal	Rt	No	Wildtype	Yes	Strong	10.0	2.3	Yes
5	48	F	Parietal	Lt	Yes	Wildtype	Yes	Strong	14.4	0.6	Yes
6	42	M	Frontal	Rt	No	Wildtype	Yes	Strong	14.7	0.5	Yes
7	53	M	Parietal	Lt	No	Wildtype	Yes	Strong	11.9	2.4	Yes
8	57	F	Parietal	Rt	No	Wildtype	Yes	Strong	13.1	0.7	Yes
9	75	F	Parietal	Lt	No	Wildtype	Yes	Strong	12.2	0.8	Yes
10	39	M	Parietal	Lt	No	Wildtype	Yes	Strong	12.0	0.5	No
11	68	M	Temporal	Rt	No	Wildtype	Yes	Vague	11.1	2.4	Yes
12	80	M	Parietal	Lt	No	Wildtype	Yes	Strong	7.4	1.0	Yes
13	61	M	Temporal	Lt	No	Wildtype	Yes	Strong	4.2	1.5	No
14	67	M	Frontal	Lt	No	Wildtype	Yes	Strong	13.4	0.5	No
15	62	M	Temporal	Rt	No	Wildtype	Yes	Vague	7.7	3.4	No
16	69	M	Temporal	Lt	Yes	Wildtype	Yes	Vague	12.3	1.4	No
17	58	M	Central	Lt	Yes	Wildtype	Yes	Strong	12.1	0.6	No
18	70	F	Parietal	Rt	No	Wildtype	Yes	Strong	12.2	2.1	No
19	47	F	Parietal	Rt	No	Wildtype	Yes	Strong	10.0	2.3	No
20	66	M	Frontal	Lt	No	Wildtype	Yes	Strong	8.4	1.9	Yes
21	59	F	Parietal	Rt	No	Wildtype	Yes	Strong	10.3	1.8	No
22	52	M	Temporal	Rt	No	Mutated	Yes	Strong	13.7	0.3	Yes
23	32	F	Frontal	Lt	Yes	Mutated	Yes	Strong	11.6	1.2	Yes
24	51	F	Temporal	Rt	No	Wildtype	Yes	Strong	11.8	1.8	No
25	54	F	Frontal	Lt	Yes	Wildtype	Yes	Strong	11.5	1.6	No
26	72	F	Temporal	Rt	Yes	Wildtype	Yes	Strong	11.4	2.4	Yes
27	45	F	Frontal	Rt	Yes	Wildtype	Yes	Strong	9.5	1.1	No
28	54	M	Parietal	Lt	No	Wildtype	Yes	Strong	11.1	1.8	Yes
29	61	M	Parietal	Rt	Yes	Wildtype	Yes	Strong	11.8	0.7	Yes
30	71	F	Temporal	Rt	No	Wildtype	Yes	Strong	12.2	2.7	No
31	27	F	Frontal	Lt	Yes	Mutated	Yes	Strong	12.5	0.5	No
32	66	F	Frontal	Lt	No	Wildtype	Yes	Strong	11.1	1.7	No
33	67	M	Frontal	Rt	No	Wildtype	Yes	Vague	14.5	0.7	Yes
34	56	M	Temporal	Rt	Yes	Wildtype	Yes	Strong	11.9	0.6	No
35	52	F	Parietal	Rt	Yes	Wildtype	Yes	Strong	13.4	1.3	No
36	42	M	Temporal	Rt	No	Wildtype	Yes	Strong	14.5	0.3	Yes
37	72	M	Frontal	Lt	No	Wildtype	Yes	Strong	13.4	1	No
38	57	M	Parietal	Rt	No	Wildtype	Yes	Strong	13.9	0.8	Yes
39	44	M	Insular	Rt	Yes	Mutated	Yes	Strong	13.3	0.7	Yes
40	51	M	Temporal	Rt	Yes	Wildtype	Yes	Strong	12.2	1.7	No
41	68	M	Temporal	Rt	No	Wildtype	Yes	Strong	13.6	1.2	Yes
42	49	F	Temporal	Rt	No	Wildtype	Yes	Strong	9.7	2	Yes
43	38	F	Parietal	Rt	No	Wildtype	Yes	Strong	14.1	0.3	Yes
44	59	F	Parietal	Lt	No	Wildtype	Yes	Strong	14	0.2	Yes
45	46	M	Frontal	Lt	No	Mutated	Yes	Strong	13.5	0.4	No
46	59	F	Central	Lt	No	Wildtype	Yes	Strong	14.5	0.3	Yes
47	66	M	Frontal	Lt	No	Wildtype	Yes	Strong	12.6	2.3	Yes

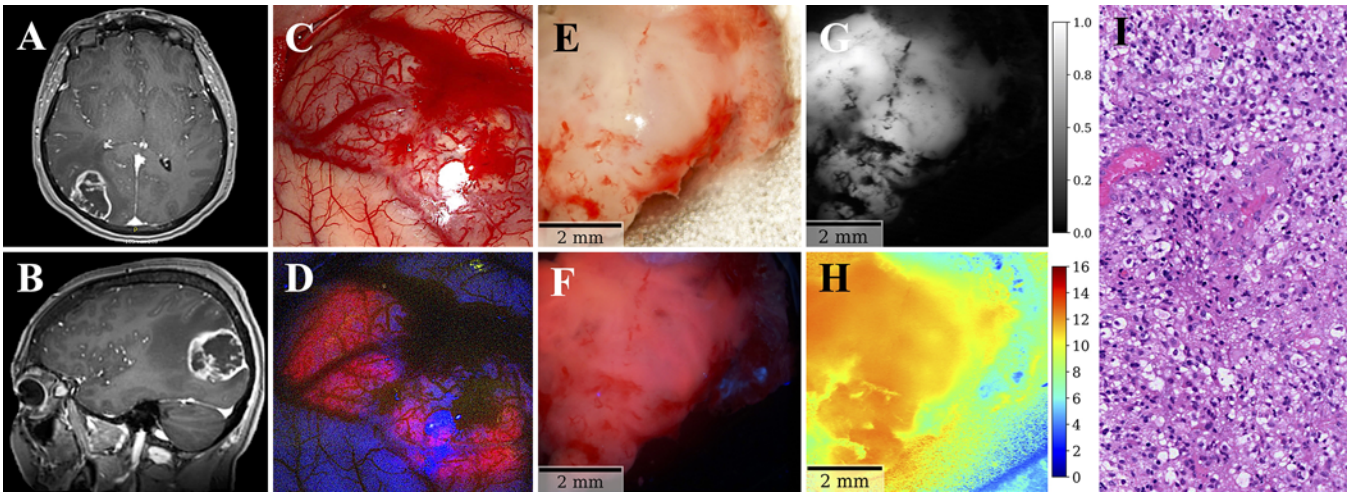

FIG. 1. Illustrative case of a 59-year-old patient after surgery of a GBM and no preoperative treatment with dexamethasone. Axial (**A**) and sagittal (**B**) T1-weighted MRI sequences show a right, occipital tumor with significant ringlike CE. Intraoperative images under conventional white light (**C**) show a superficial tumor suspected area that was confirmed under blue light (**D**) showing strong fluorescence without preoperative intake of dexamethasone. A representative sample from the tumor with strong fluorescence was analyzed by FLIM, and again images under white light (**E**) and blue light (**F**) showing strong fluorescence were obtained under the microscope. Demodulated fluorescence intensity (relative units) of the tumor sample, measured with the raster-scanning FLIM system (**G**). The FLIM is color coded, and a mean lifetime value of 10.3 nsec was measured across the sample (**H**). The histological diagnosis of a GBM WHO grade IV, IDH wildtype, and MGMT methylated, was confirmed by neuropathology (**I**). H&E, original magnification ×100. Figure is available in color online only.

to clarify if dexamethasone pretreatment is required in routine clinical practice to achieve optimal fluorescence effect in fluorescence-guided surgery of GBMs.

Present Study

Therefore, we designed the present study to objectively evaluate the influence of dexamethasone on visible fluorescence/PpIX accumulation measured by quantitative FLIM technique in representative tissue samples from the compact tumor core of patients with GBMs. Thus, we were able to minimize the interobserver variability of conventional classification of visible 5-ALA fluorescence and the

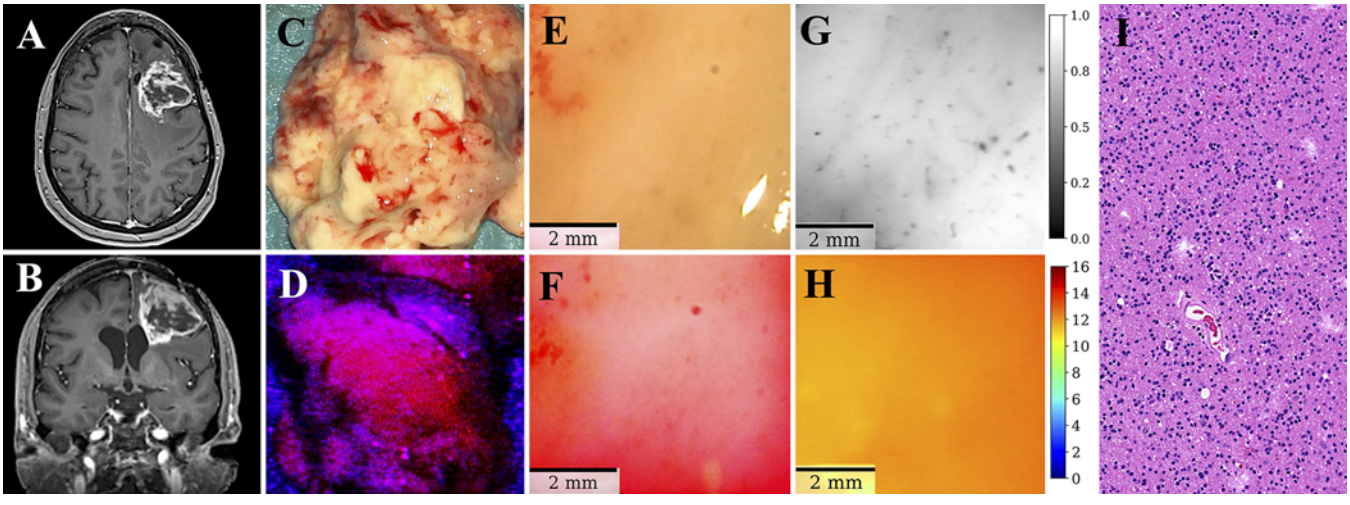

FIG. 2. Illustrative case of a 32-year-old patient with a radiologically suspected GBM and preoperative intake of dexamethasone. Preoperative axial (**A**) and coronal (**B**) contrast-enhanced T1-weighted MR images show a left frontal intraaxial tumor with significant ringlike CE. During surgery, images under white light (**C**) and under blue light (**D**) were obtained showing strong fluorescence. Immediately after surgery, images of a representative fluorescent tumor sample were obtained under white light (**E**) and under blue light (**F**) to confirm positive fluorescence. Demodulated fluorescence intensity (relative units) of the tumor sample, measured with the raster-scanning FLIM system (**G**). The FLIM is color coded, and a mean lifetime value of 11.6 nsec was measured across the sample (**H**). The histological diagnosis of a GBM WHO grade IV, IDH1 mutated, MGMT nonmethylated, was confirmed by neuropathology (**I**). H&E, original magnification ×100. Figure is available in color online only.

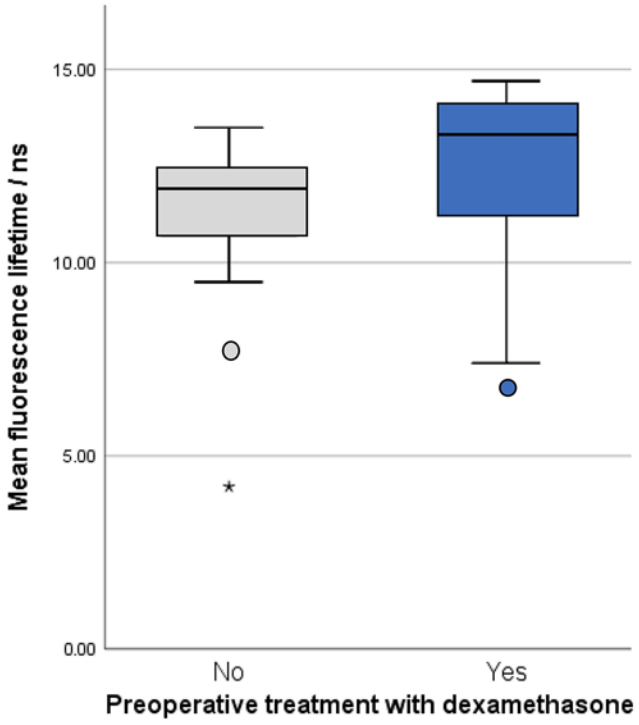

FIG. 3. Correlation of dexamethasone intake and PpIX accumulation measured by FLIM showed no statistically significant influence according to the mean FLIM values of all 47 patients. Figure is available in color online only.

subjective evaluation of the operating surgeon by using this approach.

Influence of Dexamethasone on Visible Fluorescence and PpIX Fluorescence Lifetime

Rapid tumor growth is a common feature in patients suffering from GBM, resulting frequently in vasogenic edema and increased intracranial pressure.[35–37] Therefore, the perioperative medical treatment with corticosteroids is commonly applied in these patients to relieve clinical symptoms and to improve quality of life.[38–41] In our study, preoperative dexamethasone treatment was applied exclusively in patients presenting with significant clinical symptoms. Despite the absence of preoperative dexamethasone treatment, visible 5-ALA fluorescence was observed during surgery in all patients (n = 20). Additionally, we did not find a statistically significant difference in intratumoral PpIX accumulation in patients with and without preoperative treatment with dexamethasone. To our knowledge, this represents the first study demonstrating that preoperative dexamethasone treatment has no significant impact on the 5-ALA–induced PpIX accumulation in patients with GBMs. Our current preliminary data thus indicate that the pretreatment with dexamethasone is not obligatory before fluorescence-guided surgery in GBMs.

Influence of Dexamethasone in Different Tumor Entities

In recent years, the potential influence of corticosteroids on 5-ALA fluorescence was investigated in other intracranial tumor entities and dermatological diseases. In this sense, Kiesel et al. investigated the value of 5-ALA in stereotactic biopsies of 41 intracranial lymphomas and found no influence of preoperative intake of corticosteroids on the intraoperative fluorescence effect.[42] In agreement with those findings, our group observed no influence of treatment with dexamethasone prior to surgery on the intraoperative 5-ALA fluorescence status in a cohort of 110 suspected low-grade gliomas.[11] In dermatology, Wiegell et al. investigated the influence of topical corticosteroid treatment on 5-ALA–induced photodynamic therapy in multiple mild actinic keratoses and reported that additional use of corticosteroids did not affect the efficiency of the photodynamic therapy.[43,44]

Study Limitations

First, the present study includes only a relatively small number of samples derived from surgery of GBMs. Therefore, future studies analyzing the influence of dexamethasone on 5-ALA–induced PpIX accumulation comprising a larger number of patients are needed to confirm our preliminary data. Second, we were not able to provide a detailed analysis of the duration and the total daily dose of corticosteroid intake and 5-ALA fluorescence because only 4 patients received dexamethasone for more than 7 days, and patients received a large variety of different corticosteroid doses, respectively. Thus, larger studies including also patients with long-term corticosteroid treatment and larger patient subgroups with the same total daily dose of dexamethasone are needed. Furthermore, we did not match patients of each group according to age, comorbidities, or tumor size in our current study due to the relatively small number of patients in each subgroup. Finally, in our study we analyzed only one representative sample from the tumor core in each GBM. However, we did not investigate the influence of dexamethasone on the 5-ALA–induced PpIX accumulation in other intratumoral areas outside the tumor core, such as the infiltration zone or the tumor margin. Future studies analyzing multiple tissue samples from outside the tumor core (strong fluorescence) with lower 5-ALA–induced PpIX accumulation (vague or no fluorescence), including the infiltrative tumor region as well as tumor margin, are warranted for comparison of patients with GBMs with and without dexamethasone pretreatment prior to surgery for quantitative PpIX FLIM analysis.

Conclusions

In our study, we evaluated for the first time the influence of dexamethasone pretreatment on visible 5-ALA–induced fluorescence and PpIX accumulation measured by FLIM in a series of patients with GBMs. According to our preliminary data, we found no significant influence of dexamethasone intake on visible 5-ALA fluorescence during surgery as well as PpIX accumulation based on FLIM. Consequently, our data indicate that pretreatment with dexamethasone is not obligatory prior to 5-ALA fluorescence-guided surgery in GBM, and we thus recommend that dexamethasone should be applied before these procedures only when clinically indicated.

References

1. Sanai N, Berger MS. Glioma extent of resection and its impact on patient outcome. *Neurosurgery*. 2008;62(4):753-764, 264-266.
2. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol*. 2006; 7(5):392-401.
3. Almeida JP, Chaichana KL, Rincon-Torroella J, Quinones-Hinojosa A. The value of extent of resection of glioblastomas: clinical evidence and current approach. *Curr Neurol Neurosci Rep*. 2015;15(2):517.
4. Stummer W, Reulen HJ, Meinel T, Pichlmeier U, Schumacher W, Tonn JC, et al. Extent of resection and survival in glioblastoma multiforme: identification of and adjustment for bias. *Neurosurgery*. 2008;62(3):564-576.
5. Hervey-Jumper SL, Berger MS. Maximizing safe resection of low- and high-grade glioma. *J Neurooncol*. 2016;130(2): 269-282.
6. Stummer W, Stocker S, Wagner S, Stepp H, Fritsch C, Goetz C, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid-induced porphyrin fluorescence. *Neurosurgery*. 1998;42(3):518-526.
7. Tonn JC, Stummer W. Fluorescence-guided resection of malignant gliomas using 5-aminolevulinic acid: practical use, risks, and pitfalls. *Clin Neurosurg*. 2008;55:20-26.
8. Hadjipanayis CG, Widhalm G, Stummer W. What is the surgical benefit of utilizing 5-aminolevulinic acid for fluorescence-guided surgery of malignant gliomas? *Neurosurgery*. 2015;77(5):663-673.
9. Lakomkin N, Hadjipanayis CG. Fluorescence-guided surgery for high-grade gliomas. *J Surg Oncol*. 2018;118(2):356-361.
10. Widhalm G, Minchev G, Woehrer A, Preusser M, Kiesel B, Furtner J, et al. Strong 5-aminolevulinic acid-induced fluorescence is a novel intraoperative marker for representative tissue samples in stereotactic brain tumor biopsies. *Neurosurg Rev*. 2012;35(3):381-391.
11. Wadiura LI, Mischkulnig M, Hosmann A, Borkovec M, Kiesel B, Rötzer T, et al. Influence of corticosteroids and antiepileptic drugs on visible 5-aminolevulinic acid fluorescence in a series of initially suspected low-grade gliomas including World Health Organization grade II, III, and IV gliomas. *World Neurosurg*. 2020;137:e437-e446.
12. Kiesel B, Mischkulnig M, Woehrer A, Martinez-Moreno M, Millesi M, Mallouhi A, et al. Systematic histopathological analysis of different 5-aminolevulinic acid-induced fluorescence levels in newly diagnosed glioblastomas. *J Neurosurg*. 2018;129(2):341-353.
13. Mischkulnig M, Kiesel B, Borkovec M, Wadiura LI, Benner D, Hosmann A, et al. High interobserver agreement in the subjective classification of 5-aminolevulinic acid fluorescence levels in newly diagnosed glioblastomas. *Lasers Surg Med*. 2020;52(9):814-821.
14. Erkkilä MT, Reichert D, Hecker-Denschlag N, Wilzbach M, Hauger C, Leitgeb RA, et al. Surgical microscope with integrated fluorescence lifetime imaging for 5-aminolevulinic acid fluorescence-guided neurosurgery. *J Biomed Opt*. 2020; 25(7):1-7.
15. Erkkilä MT, Bauer B, Hecker-Denschlag N, Madera Medina MJ, Leitgeb RA, Unterhuber A, et al. Widefield fluorescence lifetime imaging of protoporphyrin IX for fluorescence-guided neurosurgery: An ex vivo feasibility study. *J Biophotonics*. 2019;12(6):e201800378.
16. Reichert D, Erkkilä MT, Holst G, Hecker-Denschlag N, Wilzbach M, Hauger C, et al. Towards real-time wide-field fluorescence lifetime imaging of 5-ALA labeled brain tumors with multi-tap CMOS cameras. *Biomed Opt Express*. 2020; 11(3):1598-1616.
17. Erkkilä MT, Reichert D, Gesperger J, Kiesel B, Roetzer T, Mercea PA, et al. Macroscopic fluorescence-lifetime imaging of NADH and protoporphyrin IX improves the detection and grading of 5-aminolevulinic acid-stained brain tumors. *Sci Rep*. 2020;10(1):20492.
18. Widhalm G, Kiesel B, Woehrer A, Traub-Weidinger T, Preusser M, Marosi C, et al. 5-Aminolevulinic acid induced fluorescence is a powerful intraoperative marker for precise histopathological grading of gliomas with non-significant contrast-enhancement. *PLoS One*. 2013;8(10):e76988.
19. Widhalm G, Wolfsberger S, Minchev G, Woehrer A, Krssak M, Czech T, et al. 5-Aminolevulinic acid is a promising marker for detection of anaplastic foci in diffusely infiltrating gliomas with nonsignificant contrast enhancement. *Cancer*. 2010;116(6):1545-1552.
20. Stummer W, Stepp H, Möller G, Ehrhardt A, Leonhard M, Reulen HJ. Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue. *Acta Neurochir (Wien)*. 1998;140(10):995-1000.
21. Berezin MY, Achilefu S. Fluorescence lifetime measurements and biological imaging. *Chem Rev*. 2010;110(5):2641-2684.
22. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, et al. The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol*. 2007;114(2): 97-109.
23. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. *Acta Neuropathol*. 2016;131(6):803-820.
24. Panciani PP, Fontanella M, Schatlo B, Garbossa D, Agnoletti A, Ducati A, Lanotte M. Fluorescence and image guided resection in high grade glioma. *Clin Neurol Neurosurg*. 2012; 114(1):37-41.
25. Stummer W, Novotny A, Stepp H, Goetz C, Bise K, Reulen HJ. Fluorescence-guided resection of glioblastoma multiforme by using 5-aminolevulinic acid-induced porphyrins: a prospective study in 52 consecutive patients. *J Neurosurg*. 2000;93(6):1003-1013.
26. Waljee AK, Rogers MAM, Lin P, Singal AG, Stein JD, Marks RM, et al. Short term use of oral corticosteroids and related harms among adults in the United States: population based cohort study. *BMJ*. 2017;357:j1415.
27. Weissman DE, Dufer D, Vogel V, Abeloff MD. Corticosteroid toxicity in neuro-oncology patients. *J Neurooncol*. 1987;5(2): 125-128.
28. Dixit KS, Kumthekar PU. Optimal management of corticosteroids in patients with intracranial malignancies. *Curr Treat Options Oncol*. 2020;21(9):77.
29. Brophy T, Chalk JB, Ridgeway K, Tyrer JH, Eadie MJ. Cortisol production during high dose dexamethasone therapy in neurological and neurosurgical patients. *J Neurol Neurosurg Psychiatry*. 1984;47(10):1081-1086.
30. Lawrence JE, Steele CJ, Rovin RA, Belton RJ Jr, Winn RJ. Dexamethasone alone and in combination with desipramine, phenytoin, valproic acid or levetiracetam interferes with 5-ALA-mediated PpIX production and cellular retention in glioblastoma cells. *J Neurooncol*. 2016;127(1):15-21.
31. Valdés PA, Jacobs V, Harris BT, Wilson BC, Leblond F, Paulsen KD, Roberts DW. Quantitative fluorescence using 5-aminolevulinic acid-induced protoporphyrin IX biomarker as a surgical adjunct in low-grade glioma surgery. *J Neurosurg*. 2015;123(3):771-780.
32. Valdés PA, Jacobs VL, Leblond F, Wilson BC, Paulsen KD, Roberts DW. Quantitative spectrally resolved intraoperative fluorescence imaging for neurosurgical guidance in brain tumor surgery: pre-clinical and clinical results. *Proc SPIE*. 2014;8928:892809.
33. Bekelis K, Valdés PA, Erkmen K, Leblond F, Kim A, Wilson BC, et al. Quantitative and qualitative 5-aminolevulinic acid-

induced protoporphyrin IX fluorescence in skull base meningiomas. *Neurosurg Focus*. 2011;30(5):E8.
34. Widhalm G, Olson J, Weller J, Bravo J, Han SJ, Phillips J, et al. The value of visible 5-ALA fluorescence and quantitative protoporphyrin IX analysis for improved surgery of suspected low-grade gliomas. *J Neurosurg*. 2020;133(1):79-88.
35. Strugar JG, Criscuolo GR, Rothbart D, Harrington WN. Vascular endothelial growth/permeability factor expression in human glioma specimens: correlation with vasogenic brain edema and tumor-associated cysts. *J Neurosurg*. 1995;83(4):682-689.
36. Hui CY, Rudra S, Ma S, Campian JL, Huang J. Impact of overall corticosteroid exposure during chemoradiotherapy on lymphopenia and survival of glioblastoma patients. *J Neurooncol*. 2019;143(1):129-136.
37. Petrelli F, De Stefani A, Ghidini A, Bruschieri L, Riboldi V, Dottorini L, et al. Steroids use and survival in patients with glioblastoma multiforme: a pooled analysis. *J Neurol*. 2021;268(2):440-447.
38. van Breemen MSM, Rijsman RM, Taphoorn MJB, Walchenbach R, Zwinkels H, Vecht CJ. Efficacy of anti-epileptic drugs in patients with gliomas and seizures. *J Neurol*. 2009;256(9):1519-1526.
39. Newton HB, Dalton J, Goldlust S, Pearl D. Retrospective analysis of the efficacy and tolerability of levetiracetam in patients with metastatic brain tumors. *J Neurooncol*. 2007;84(3):293-296.
40. Kurzwelly D, Herrlinger U, Simon M. Seizures in patients with low-grade gliomas—incidence, pathogenesis, surgical management, and pharmacotherapy. *Adv Tech Stand Neurosurg*. 2010;35:81-111.
41. Vecht C, Royer-Perron L, Houillier C, Huberfeld G. Seizures and anticonvulsants in brain tumours: frequency, mechanisms and anti-epileptic management. *Curr Pharm Des*. 2017;23(42):6464-6487.
42. Kiesel B, Millesi M, Woehrer A, Furtner J, Bavand A, Roetzer T, et al. 5-ALA-induced fluorescence as a marker for diagnostic tissue in stereotactic biopsies of intracranial lymphomas: experience in 41 patients. *Neurosurg Focus*. 2018;44(6):E7.
43. Wiegell SR, Petersen B, Wulf HC. Topical corticosteroid reduces inflammation without compromising the efficacy of photodynamic therapy for actinic keratoses: a randomized clinical trial. *Br J Dermatol*. 2014;171(6):1487-1492.
44. Wiegell SR, Petersen B, Wulf HC. Pulse photodynamic therapy reduces inflammation without compromising efficacy in the treatment of multiple mild actinic keratoses of the face and scalp: a randomized clinical trial. *Br J Dermatol*. 2016;174(5):979-984.

Disclosures

Dr. Widhalm received restricted travel grants from NX Development Corp.

Author Contributions

Conception and design: Widhalm, Wadiura, Reichert. Acquisition of data: all authors. Analysis and interpretation of data: Widhalm, Wadiura, Reichert, Roetzer, Mischkulnig. Drafting the article: Widhalm, Wadiura, Reichert. Critically revising the article: Widhalm, Wadiura, Sperl, Lang, Kiesel, Erkkilae, Woehrer, Furtner, Roetzer, Mischkulnig. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Widhalm. Statistical analysis: Roetzer, Mischkulnig. Administrative/technical/material support: Woehrer, Leitgeb. Study supervision: Widhalm.

Correspondence

Georg Widhalm: Medical University of Vienna, Austria. georg.widhalm@meduniwien.ac.at.