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Double dose of 5-aminolevulinic acid and its effect on protoporphyrin IX accumulation in low-grade glioma

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OBJECTIVE Administration of 5-aminolevulinic acid (5-ALA) does not regularly elicit fluorescence in low-grade glioma (LGG) at currently established doses and timing of administration. One explanation may be differences in blood-brain barrier (BBB) integrity compared to high-grade glioma. The authors hypothesized that for a BBB semipermeable to 5-ALA there might be a relationship between plasma 5-ALA concentration and its movement into the brain. A higher dose would elicit more 5-ALA conversion into protoporphyrin IX (PPIX). The authors present a case series of patients harboring LGG who received higher doses of 5-ALA.

METHODS Patients undergoing surgery for indeterminate glioma later diagnosed as LGG were included in this study. 5-ALA was administered at a standard dose of 20 mg/kg body weight (bw) 4 hours prior to induction of anesthesia. A subgroup of patients received a higher dose of 40 mg/kg bw. Fluorescence was evaluated visually and PPIX concentration (cPPIX) was determined ex vivo by hyperspectral measurements in freshly extracted tissue. All adverse events were recorded.

RESULTS A total of 23 patients harboring diffuse low-grade astrocytomas (n = 19) and oligodendrogliomas (n = 4) were analyzed. Thirteen patients received 20 mg/kg bw, and 10 patients received 40 mg/kg bw of 5-ALA. In the 20 mg/kg group, 30.8% (4 of 13) of tumors harbored areas of visible fluorescence, compared to 60% of cases (n = 6 of 10) with 40 mg/kg bw. The threshold to visibility was 1 μg/ml in both groups. Measured over all biopsies, the mean cPPIX was significantly higher in the double-dose group (1.8 vs 0.45 μg/ml; $p < 0.001$). In non–visibly fluorescent tissue the mean cPPIX was 0.146 μg/ml in the 20 mg/kg and 0.347 μg/ml in the 40 mg/kg group, indicating an increase of 138% ($p < 0.001$).

CONCLUSIONS These observations demonstrate different regions with different levels of PPIX accumulation in LGG. With higher 5-ALA doses cPPIX increases, leading to more regions surpassing the visibility threshold of 1 μg/ml. These observations can be explained by the fact that the BBB in LGG is semipermeable to 5-ALA. Higher 5-ALA doses result in more PPIX conversion, an observation with implications for future dosing in LGG.

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KEYWORDS 5-ALA; 5-aminolevulinic acid; fluorescence-guided resection; low-grade glioma; PPIX concentration; protoporphyrin IX; oncology

Extent of resection of tumor tissue correlates with the outcome of patients harboring malignant glioma.[1,2] Fluorescence guidance[3] is a reliable adjunct in malignant glioma surgery for maximizing extent of resection. 5-Aminolevulinic acid (or δ-aminolevulinic acid; 5-ALA), a natural heme precursor formed by the condensation of succinyl-CoA and glycine,[4] is the most established fluorophore used for high-grade glioma (HGG) surgery. Nonfluorescent 5-ALA, a naturally occurring metabolite in the heme biosynthesis pathway, elicits the expression of fluorescent protoporphyrin IX (PPIX) in HGG cells when administered externally, and can be visualized intraoperatively with the assistance of commercially available filter systems.[5–7] In the last 20 years, 5-ALA–induced fluorescence has gained a pivotal role in HGG surgery, and—supported by a multicenter phase III randomized trial[3]—was approved by the European Medical Agencies and later by the FDA.

ABBREVIATIONS 5-ALA = 5-aminolevulinic acid; ALT = alanine transaminase; AST = aspartate aminotransferase; BBB = blood-brain barrier; bw = body weight; cPPIX = PPIX concentration; CTCAE = Common Terminology Criteria for Adverse Events; FET = ^{18}F-fluoroethyltyrosine; GBM = glioblastoma; HGG = high-grade glioma; LGG = low-grade glioma; PPIX = protoporphyrin IX; ROI = region of interest; SUVmax = maximum standardized uptake value.

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In our experience, the tenets of fluorescence guidance in low-grade glioma (LGG) differ from those in HGG. Use of 5-ALA–induced fluorescence can help find malignant foci in apparent LGG, which can be selectively interrogated by histopathological analysis to avoid undergrading during histopathological examination. These foci can be found in up to 55% of tumors with imaging features of LGG.[8,9]

In histologically verified LGG, approximately 20% of biopsy tissue accumulates fluorescence, visible using the surgical microscope.[10] However, even in those LGGs without visible fluorescence, lower levels of PPIX can be measured via spectroscopy[11–13] or can be visualized using confocal microscopy.[14]

In a recent study, our group evaluated factors predicting visible fluorescence in a cohort of patients harboring only LGG. We concluded that Gd enhancement, Ki-67/MIB-1 index, ^{18}F-fluoroethyltyrosine (FET) PET maximum standardized uptake value (SUVmax), and apparent diffusion coefficient–based tumor cellularity significantly increased the probability of intraoperative visible fluorescence.[13] However, the clinical value and efficacy of fluorescence guidance in LGG are still under scientific scrutiny.

The reasons for the poor accumulation of visible PPIX in LGG tissue are unclear. A number of factors have been discussed that might principally influence accumulation in gliomas, such as proliferation and metabolic characteristics[15–17] or tumor microenvironment, e.g., hypoxia, pH, and temperature.[18,19] In addition, tumor cell density, which has been correlated with visible fluorescence,[20,21] varies between HGGs and LGGs.

We retrospectively tested this hypothesis in a series of patients with indeterminate gliomas treated with 40 mg/kg of 5-ALA in a compassionate use setting, analyzing tissue PPIX concentration (cPPIX) and the frequency of visible fluorescence. Recently published data have demonstrated higher doses of 5-ALA to be safe.[22] To the best of our knowledge, this is the first study evaluating administration of a higher 5-ALA dose in LGG.

Methods

Patients undergoing surgery for gliomas of indeterminate grade, which were later diagnosed as LGG, at the Department of Neurosurgery, University Hospital of Münster, were included in this study. Indications for surgery were discussed in an interdisciplinary tumor board meeting.

The 5-ALA (Gliolan; medac) was administered at a dose of 20 mg/kg body weight (bw) 4 hours prior to induction of anesthesia. A subset of patients received a higher dose of 40 mg/kg bw, likewise at 4 hours prior to induction of anesthesia. Patients were treated with the higher doses on a compassionate use basis. Patient selection for the double-dose group was performed on an individual basis after discussion with patients. HGGs were not included in this analysis.

During surgery, an OPMI Pentero microscope equipped with the BLUE400 filter system for PPIX visualization (Carl Zeiss Meditec) was used to visualize fluorescence. The WHO classification of 2016[23] provided the guidelines to assess all tumor biopsies included in this study. During surgery, tissues identified as tumor were collected and protected from light exposure. If available, tumor samples were drawn from the tumor area of strongest fluorescence, with tissues being mostly homogeneous regarding fluorescence pattern.

Liver enzymes, i.e., serum aspartate aminotransferase (AST) and alanine transaminase (ALT), and gamma-glutamyl transferase (Gamma-GT), were evaluated prior to and after surgery, as is standard at our institution. The Common Terminology Criteria for Adverse Events (CTCAE; version 5.0) for hepatobiliary disorders was used to categorize any adverse events regarding the liver function.

Written informed consent was obtained from each patient. 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. Data collection and scientific use of biopsies had previously been approved by the ethics committee of the University of Münster.

Hyperspectral Measurements

Fluorescence intensity measurements from tumor biopsies were performed using a hyperspectral imaging system, as previously described.[24] At least 3 biopsies were analyzed during surgery by using hyperspectral measurements. Spectra from white light and fluorescence were captured in ten 10 × 10–pixel regions of interest (ROIs) per tumor sample. The intensities of the pixels within each ROI were averaged to give a final spectrum for each ROI. An empirically derived exponent, together with normalization factors obtained from the white-light intensity in diverse spectral regions, assisted normalization of the fluorescence spectra for inhomogeneous scattering and absorption properties throughout the tissue.[25] The PPIX spectrum was isolated from the measured signal by calculating the least-squares solution to an overdetermined linear system of equations, which provides the relative PPIX fluorescence intensity. Moreover, with the help of fluorescence phantoms with known cPPIX, our algorithm was calibrated in order to indirectly estimate cPPIX in our biopsies. Even though we refer to the presented values as concentrations, "contribution to the PPIX spectra" would be a more precise description given the imperfect calibration.[45] Nevertheless, the values provided by this algorithm are precise and self-consistent, so relative differences in this study are reliable. However, absolute cPPIX may differ from that found by other groups that perform similar experiments with other devices. This analysis was carried out with commercially available software (MATLAB; The Math Works, Inc.).

Statistical Analysis

Calculations were performed using commercially available software (MATLAB). The p values were calculated using the nonparametric Wilcoxon rank-sum test. A p value < 0.05 was considered statistically significant. All

TABLE 1. Demographic data in 23 patients with LGGs

	5-ALA Dose	
	Single, 20 mg/kg bw	Double, 40 mg/kg bw
No. of pts	13	10
Age (median ± SD)	49 ± 11.9	35.5 ± 9.2
Sex		
Female	6 (46.2%)	6 (60%)
Male	7 (53.8%)	4 (40%)
Recurrent tumor	6 (46.2%)	3 (30%)
Histology (WHO 2016 classification)		
Diffuse astrocytoma, IDH wild type	10 (76.9%)	8 (80%)
Diffuse astrocytoma, IDH mutant	0	1 (10%)
Oligodendroglioma, IDH mutant, 1p/19q co-deleted	3 (23.1%)	1 (10%)
MRI enhancement		
+	4 (30.8%)	1 (10%)
−	9 (69.2%)	9 (90%)
FET PET (SUVmax >1.85)*		
+	8 (66.7%)	9 (90%)
−	4 (33.3%)	1 (10%)
Elevation of liver enzymes (CTCAE grade 1)		
+	4 (30.8%)	2 (20%)
−	9 (69.2%)	8 (80%)

Pts = patients.
None of the presented values were statistically significant at $p < 0.05$.
* In 1 patient in the single-dose group an FET PET diagnosis was not performed preoperatively.

TABLE 2. Visual fluorescence assessed by the surgeon and measured fluorescence intensity and PPIX values

	Single Dose	Double Dose	% Increase	p Value
Pts harboring visible fluorescence (%)	30.8%	60%	95%	0.182
Mean of max FI values (au)	0.38	0.8	110%	0.174
% of tumors w/ low to medium cPPIX (0.5–12 μg/ml)	21%	40%	90%	<0.001
Overall mean cPPIX	0.45 μg/ml	1.8 μg/ml	300%	<0.001
Mean cPPIX of weakly fluorescing tumors (≤1 μg/ml)	0.146 μg/ml	0.347 μg/ml	138%	<0.001

au = arbitrary units; FI = fluorescence intensity; max = maximum.

p values reported are 2-sided. Fluorescence quality, MIB-1 index, and cPPIX-adjusted multivariate analysis were performed using logistic regression and characterized by p values and 95% confidence intervals.

Results

Patient Cohorts and Laboratory Analysis

A total of 23 patients were included in this analysis. Thirteen (56.6%) patients received a standard of 20 mg/kg bw of 5-ALA (single dose), whereas 10 (43.4%) patients received 40 mg/kg bw (double dose; Table 1). The mean age in the single-dose cohort was 43 years and in the double-dose group it was 34.3 years (not significant). Histological findings, ^{18}F-FET PET uptake, and Gd contrast enhancement in MRI did not differ significantly between patients treated with different 5-ALA doses (Table 1). We did not observe any relation or significant difference between measured cPPIX, the probability of intraoperative visible fluorescence, and calculated SUVmax in PET. The mean SUVmax was 2.99 in double-dose and 2.81 in the single-dose patients (not significant), and therefore comparable.

No clinical sequelae related to the 5-ALA dosage were observed, nor was there a significant difference in the measurements of liver enzymes between the two groups. Specifically, we did not observe any higher skin photosensitivity in the early follow-up evaluation. Only 2 (20%) asymptomatic patients in the double-dose group demonstrated mildly elevated AST values (55 and 132 U/L; reference range 10–35, CTCAE grade 1–2 toxicity), with one of them furthermore presenting a mildly elevated ALT value (134 U/L; reference range 10–35, CTCAE grade 2 toxicity) on the first day after surgery, all of which completely resolved 10 days after surgery. In the single-dose group, 4 (30.1%) asymptomatic individuals demonstrated mildly elevated AST values (range 42–63 U/L, CTCAE grade 1 toxicity), which likewise normalized 10 days after surgery (Table 1).

Visible Fluorescence and Hyperspectral Measurements

In the single-dose group, 4 (30.8%) patients' tumors harbored visible fluorescence, whereas 60% (6 of 10) displayed intraoperative visible fluorescence in the double-dose group. This demonstrates a numerical increase of 95% ($p = 0.182$; Table 2).

We noted a direct relationship between fluorescence that could be perceived visually using the surgical microscope and cPPIX. With minor exceptions, we found values lower than 1 μg/ml not to be visible to the eye (Fig. 1). Furthermore, the calculated cPPIX was significantly correlated with visible fluorescence (Fig. 1, Table 3).

Overall, we observed that most pixels represented a cPPIX below 1 μg/ml. Furthermore, a trend to higher concentrations within this range of below 1 μg/ml was seen in the double-dose group. To further elucidate this difference, we created a histogram focusing pixels in this low concentration range. We observed a clear shift to the right in the double-dose group (Fig. 2).

When all samples with visible and nonvisible fluorescence were combined, the mean cPPIX was significantly higher overall in the double-dose group (1.8 μg/ml) compared to the single-dose group (0.45 μg/ml, $p < 0.001$), indicating a 300% increase (Table 2). Subsequently, we focused specifically on cPPIX in samples with concentrations ≤ 1 μg/ml (i.e., below the visibility threshold) and an-

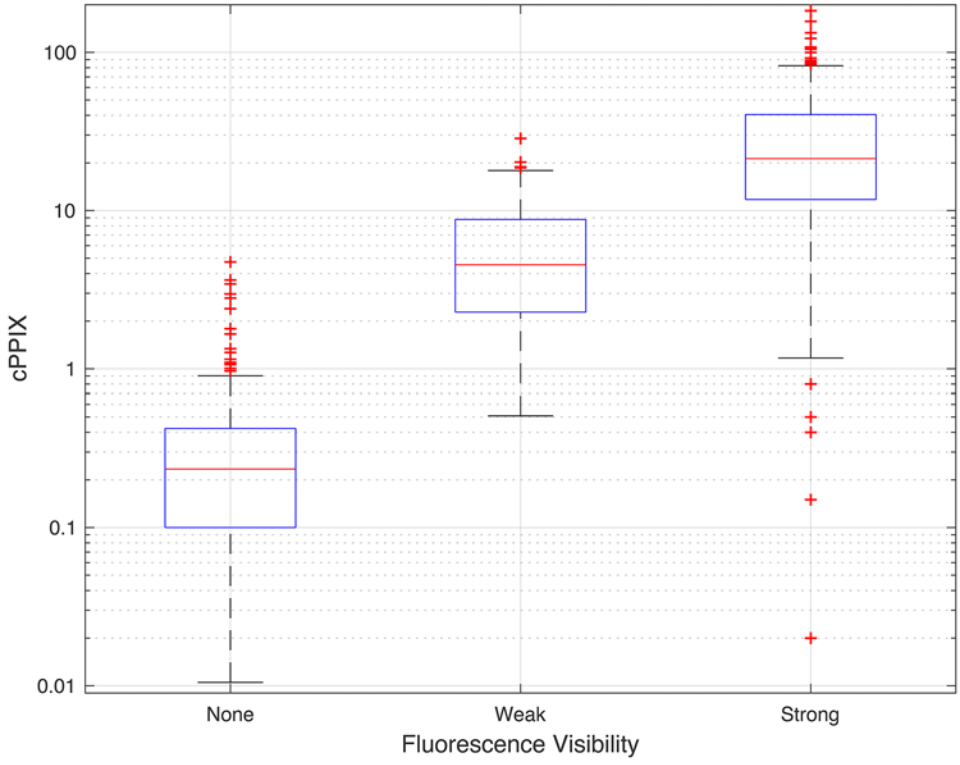

FIG. 1. Boxplot demonstrating the relationship between intraoperative visible fluorescence and measured cPPIX via spectroscopy. The majority of tissues with visible fluorescence presented a cPPIX of > 1 μg/ml. Note logarithmic ordinate. The *horizontal bars* represent medians, the *boxes* 25% and 75% percentiles, the *whiskers* 10% and 90% percentiles, and the "+" symbols represent outliers. Figure is available in color online only.

alyzed the distributions of cPPIX in these samples (Fig. 2). The cPPIX at the double-dose histogram peak was 0.347 μg/ml, and therefore twice as high as in the single-dose group (0.146 μg/ml, $p < 0.001$; Fig. 2).

The percentage of pixels in high-concentration regions was not significantly different between both groups (Fig. 3 left). However, when excluding outliers with the highest concentration, i.e., above 20 μg/ml, the percentage of pixels in the low- to medium-concentration range (0.5–12 μg/ml) differed significantly between both groups ($p < 0.001$; Fig. 3 right). Overall, 21% of tumors in the single-dose group presented with low to medium cPPIX, whereas this was the case in 40% of tumors in the double-dose group, indicating an almost 2-fold increase ($p < 0.001$; Table 2).

Overall, cPPIX was significantly higher in visibly fluorescent tissue than in nonfluorescent tissue ($p < 0.001$; Table 3). Furthermore, the MIB-1 index significantly differed among intraoperative observed visible fluorescence (none, weak, strong) among all patients, but also in both the single- and double-dose group individually ($p < 0.001$; Table 3). A multivariate regression analysis demonstrated MIB-1 index and cPPIX as independent variables for predicting fluorescence quality (Table 3).

Discussion

The poor prognosis of gliomas is mainly correlated to the infiltrative growth of these tumors. Whereas in glioblastoma (GBM) neovascularization leads to disruption of the blood-brain barrier (BBB), which allows passage of macromolecules,[17,26] in LGG the BBB is in most cases only slightly perturbed. In GBM, a disrupted BBB facilitates the formation of vasogenic edema, which can contribute to high intracranial pressure.[27] At the same time, under these conditions, it is easy for a small amino acid, such as 5-ALA, to reach these tumors. Aquaporin-4, a water channel located at the astrocyte end feet at the BBB,[27] also plays an important role in vasogenic edema formation. Its expression has been reported to correlate with the malignancy grade in gliomas.[28] Another feature that correlates with visible fluorescence is tumor cellularity,[20,21] which is high in GBM but can be low in LGG.

Based on the assumption that more selective BBB permeability in LGG is a main limiting factor for movement of 5-ALA across the BBB to glioma cells, we hypothesized that by increasing its concentration in blood, interstitial 5-ALA concentration would increase, resulting in more PPIX synthesis and accumulation.

So far a number of studies addressing 5-ALA dose have been presented, albeit all in malignant glioma. In a randomized, prospective, dose-escalation study, different doses of 0.2, 2, and 20 mg/kg bw were studied in patients with malignant glioma.[21] The authors aimed to investigate the clinical efficacy of lower but not higher doses and observed clinically significant fluorescence at 20 mg/kg bw. This standard of 20 mg/kg bw was initially chosen em-

TABLE 3. Univariate and multivariate analysis between fluorescence quality (none, weak, strong), cPPIX, and MIB-1 proliferation index

	Mean cPPIX in μg/ml (95% CI)	p Value	Mean MIB-1 Index in % (95% CI)	p Value
Univariate analysis				
All pts (n = 23)	1.29		5.63	
Visible fluorescence				
None (n = 13, 57%)	0.29		4.70	
Weak (n = 7, 30%)	2.45	<0.001	9.46	<0.001
Strong (n = 3, 13%)	9.27		5.50	
Single dose (n = 13)	0.45		5.49	
Visible fluorescence				
None (n = 9, 69%)	0.23		4.40	
Weak (n = 3, 23%)	1.25	<0.001	9.60	<0.001
Strong (n = 1, 7.7%)	27.5		9.00	
Double dose (n = 10)	1.81		5.63	
Visible fluorescence				
None (n = 4, 40%)	0.36		5.05	
Weak (n = 4, 40%)	4.07	<0.001	9.27	<0.001
Strong (n = 2, 20%)	5.63		4.80	
Multivariate analysis				
All pts (n = 23)	(0.0837–0.1225)	<0.001	(0.0224 to 0.0625)	<0.001
Single dose (n = 13)	(0.0493–0.0782)	<0.001	(0.0496 to 0.0853)	<0.001
Double dose (n = 10)	(0.1603–0.2416)	<0.001	(−0.0112 to 0.0505)	0.21

Multivariate linear regression coefficients, analyzing the simultaneous effect of cPPIX and MIB-1 index on the fluorescence visibility. The 95% CIs on the regression coefficients are given, as are their p values, for the single- and double-dose cohorts separately, and for all patients together. Note that the numbers and percentages are expressed in terms of the number of patients. Each patient had multiple biopsies, each with multiple pixels selected, so the statistics (p values, averages, etc.), which are calculated by pixel or biopsy, are stronger than they appear when just looking at the patient numbers.

pirically and has been used in most published studies and especially in those that led to the approval of 5-ALA as a surgical adjunct in HGG surgery. Since then, the dose of 20 mg/kg bw has been adopted as standard dosage.

In a dose-escalation study, a dose of up to 50 mg/kg bw was proven to be safe in HGG surgery.[22] In this cohort, patients with higher doses were more likely to present stronger visible fluorescence, suggesting a correlation.

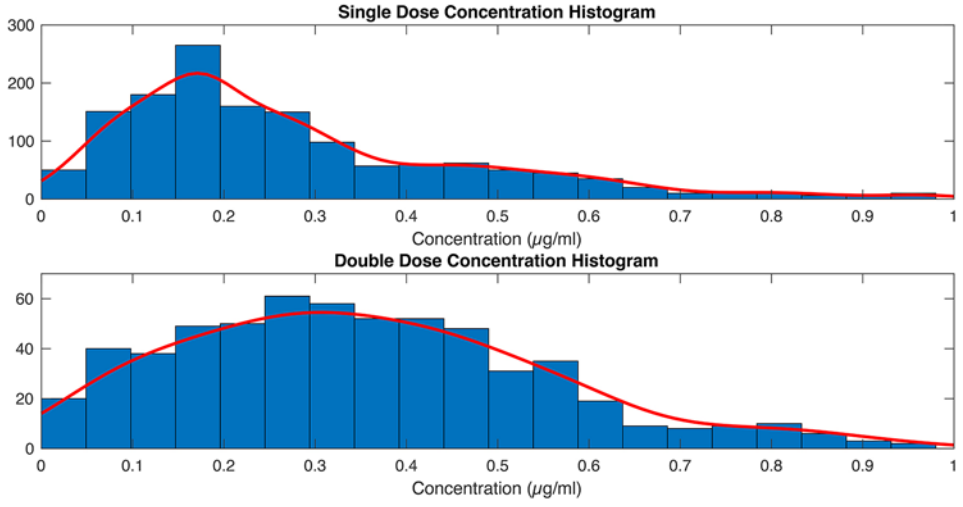

FIG. 2. Histogram for cPPIX < 1 μg/ml in evaluated biopsies of both single- and double-dose cohorts demonstrating a rightward shift in concentration maximum in the double-dose group. Values on the y-axis represent the number of pixels measured with the related concentration. Figure is available in color online only.

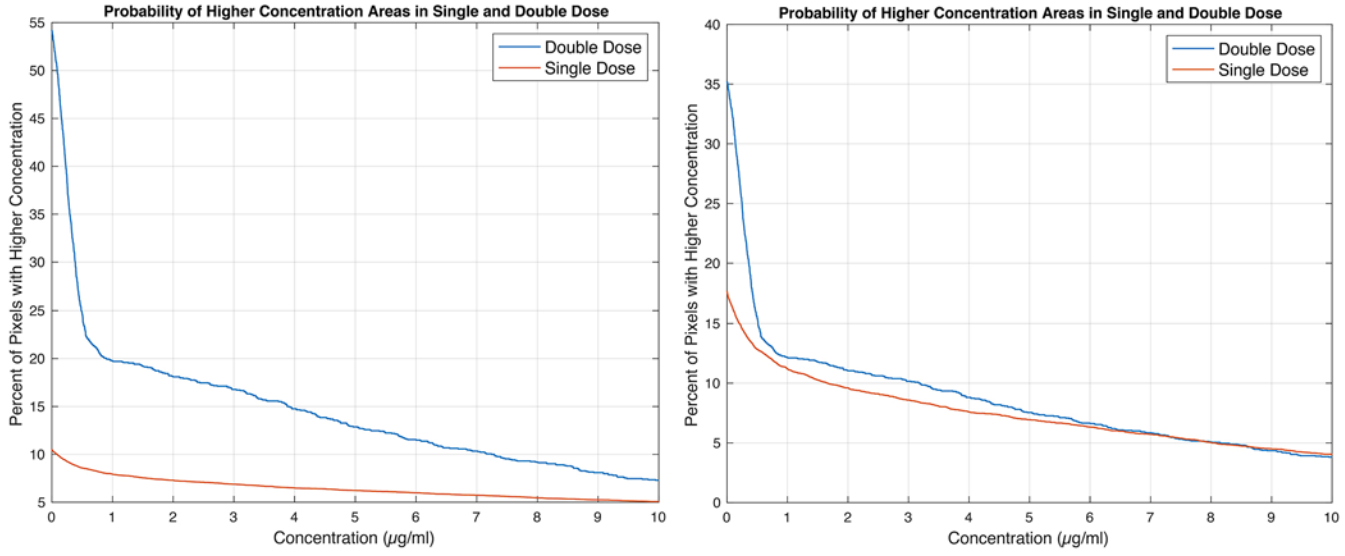

FIG. 3. Probability of higher concentration areas in the single- and double-dose group under consideration of all pixels (**left**) and after excluding every measured pixel above 20 μg/ml (**right**). Figure is available in color online only.

However, it is unclear if this relation is truly linear or subjected to a saturation curve. A further study demonstrated no significant difference in residual tumor tissue after low (10–30 mg/kg bw) versus high (40–50 mg/kg bw) doses of 5-ALA in GBM surgery.[29] Additionally, Webber et al. demonstrated, after analyzing pharmacokinetics of 5-ALA–induced PPIX expression in plasma, the safety of a dosage of up to 60 mg/kg bw in humans.[30–32] Therefore, for our cohort of patients, who were treated in an off-label setting, a dose of 40 mg/kg bw appeared to be safe.

Interestingly, in most studies applying different doses we observe similar findings: higher doses of 5-ALA tend to induce stronger fluorescence.[21,22,33] However, none of these investigations have so far studied the nuances of different doses in LGG complementing intraoperative visually observed fluorescence by performing hyperspectral measurements of acquired tissues and categorizing the frequency of high- or low-fluorescence areas, as we did in this study. As mentioned above, it is still unclear if this relationship is truly linear, subjected to a saturation curve, or somewhat related to tumor characteristics. To date, we know that molecular and pathological subgroups of gliomas will define future classifications, rather than the WHO grades per se.[23,34] Genetic subgrouping of gliomas can help us understand the different clinical courses of similarly graded tumors.[34] In the same manner, visible 5-ALA–induced fluorescence has been stated as a marker for earlier malignant transformation and has been correlated with worse prognosis in LGG.[10]

Visible Fluorescence

By doubling the 5-ALA dose in our patient cohort, we observed a 95% increase in intraoperative visible fluorescence (Table 2). This calculation was not statistically significant. We attribute this, however, to our cohort size. For hyperspectral measurements, we acquired at least 3 biopsies per patient and 10 spectra per biopsy, and thus could perform analyses using the information of hundreds of acquired pixels.

As delineated below, we observed an increase of cPPIX predominantly in low- to medium-concentration (0.5–12 μg/ml) tumor areas, whereas high-concentration regions appeared similarly frequent in both groups regardless of the administered dose. One could tentatively postulate from this observation that certain tumors are inherently permeable and after a certain point undergo a saturation of 5-ALA uptake, ergo PPIX accumulation. Hence, in these types of tumors a higher dose probably will not lead to higher frequency of intraoperatively visible fluorescence. Further evidence supports this hypothesis, because lower cPPIX was observed in strongly fluorescing biopsied tissues in the double-dose compared to the single-dose group (5.63 vs 27.5 μg/ml; Table 3). On the other hand, it appears that certain tumors are less permeable. In this case, by increasing the 5-ALA dose, we observed a significant difference in hyperspectral measurable and visible fluorescence (Fig. 4). Furthermore, we did not observe any significant difference either among the IDH status groups or between astrocytomas and oligodendrogliomas.

Tumor Regions With cPPIX Below 1 μg/ml and Between 1 and 20 μg/ml

The cPPIX for weakly fluorescing tumors (< 1 μg/ml) in the double-dose group was on average 35% higher than in the single-dose patient group. Furthermore, the concentration at the double-dose histogram peak is 2 times the concentration at the single-dose histogram peak, indicating an almost 100% increase ($p < 0.001$; Fig. 2).

Considering the higher concentrations above 1 μg/ml, we analyzed whether a double-dose administration of 5-ALA tends to cause more of these higher-fluorescence tumor regions than in the single-dose group. Merely by

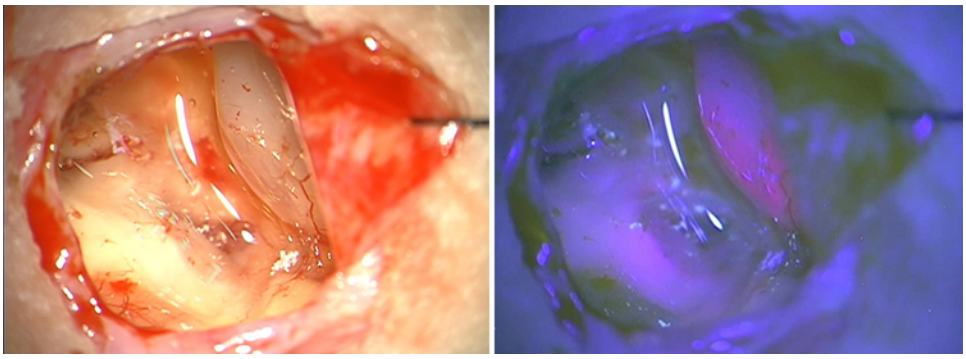

FIG. 4. Intraoperative view of a right frontal recurrent diffuse astrocytoma (IDH mutant) in a 37-year-old woman under white-light microscopy (**left**) and BLUE400 fluorescence light (**right**). A recurrence was observed in the lateral wall of the former resection cavity, and gliosis at the floor of the resection cavity likewise demonstrated weak fluorescence. Figure is available in color online only.

looking at the general distribution, it is hard to detect a difference. We therefore analyzed both concentration distributions, and for each concentration we calculated the percentage of pixels that have a higher concentration. This is analogous to the probability of a given pixel having a higher concentration. This result is plotted in Fig. 3 left. In this case, we were interested in higher concentrations. It would be reasonable to expect pixels from measurements of the double-dose group to have a higher probability of a very high cPPIX. However, in reality there is no observable or statistically significant difference between the curves.

On the other hand, ignoring the extremely high concentrations (≥ 20 μg/ml), which may be skewing the data, we see a different picture (Fig. 3 right). Indeed, although only 21% of the tumors in patients with a single dose have a concentration between 0.5 μg/ml and 12 μg/ml, 40% of the tumors in patients with a double dose fall within this range ($p < 0.001$). This is once again a 2-fold increase. Thus, we would carefully conclude that a single versus a double dose of 5-ALA makes no difference in the occurrence of extremely high-concentration tumor areas but does increase the occurrence of low to medium concentrations.

BBB Permeability

The permeability of the BBB influences 5-ALA uptake,[35,36] and one important contributing factor for distinguishing LGG and HGG appears to be the lower permeability of the BBB to 5-ALA in LGG.[26] By studying regions in the brain with and without a BBB, Olivo and Wilson demonstrated that the BBB is relatively impermeable to 5-ALA,[37] observing that the synthesis of PPIX was much higher in regions without a BBB.[37] It seems that 5-ALA can reach cells either by passive (unspecific) diffusion or by active (specific) transfer through several transporters.[38] Regarding specific uptake, a proton-dependent but Na^+-independent peptide transporter (PEPT2) has been reported to play a pivotal role in 5-ALA crossing the BBB.[39] However, other transporters have been associated with the 5-ALA uptake—the peptide transporter PEPT1, the ATP-binding cassette transporter ABCB6,[40] an amino acid transporter (PAT1), and BETA transporters.[38] Furthermore, evidence indicates that a putative Na^+/HCO_3^- organic anion transporter is also involved in this process.[39] Yet, strong scientific evidence of these transporters being responsible for a higher 5-ALA uptake in cancerous versus healthy tissue is still missing.[38] On a different note, reduced or absent ferrochelatase activity has been likewise linked to a higher PPIX accumulation in tumor cells.[41]

On the other hand, unspecific tumor vascular permeability has been correlated to the histopathological grade of gliomas,[17,26] and permeability to low-molecular-weight substances, e.g., Gd conjugates or, in analogy, 5-ALA, is lower in LGG than in HGG.[17] To this end, a hypoxic or hypoglycemic tumor microenvironment leads to increased expression of vascular endothelial growth factor, which increases both neoangiogenesis to immature vessels and unspecific permeability along the BBB.[26,42] Overall, however, the precise mechanisms of 5-ALA uptake and the extent of BBB disruption in both HGG and LGG are still an ongoing discussion.[43]

Hence, we hypothesized that specific or unspecific but selective permeability to small molecules such as 5-ALA, a molecule of only 131 Da, plays a role in the uptake into LGG tissue and may be rate limiting. In this situation, uptake depends on plasma availability of 5-ALA, which should correlate with the 5-ALA dose. Thus, an escalation of dose should result in more PPIX conversion. In HGG, in which there are massive perturbations of BBB, PPIX accumulation will be limited by the capacity of conversion rather than availability.[21,44] Additionally, in HGG we have determined a later peak of PPIX in marginal (infiltrating) tumor,[24] which we believe is supportive evidence for our hypothesis.

Based on our observations, it appears that LGG can present with either low, intermediate, or high BBB permeability (Fig. 5). In the case of low BBB permeability, tumors do not exhibit intraoperative visible fluorescence regardless of the applied dose. Here, PPIX can only be detected using spectrography. In intermediately permeable LGG, a higher dose of 40 mg/kg bw will increase frequency of intraoperative visible fluorescence. In intermediately disrupted LGG regions, a higher concentration of 5-ALA in plasma could increase its transfer through the BBB. Therefore, cPPIX will depend on the available

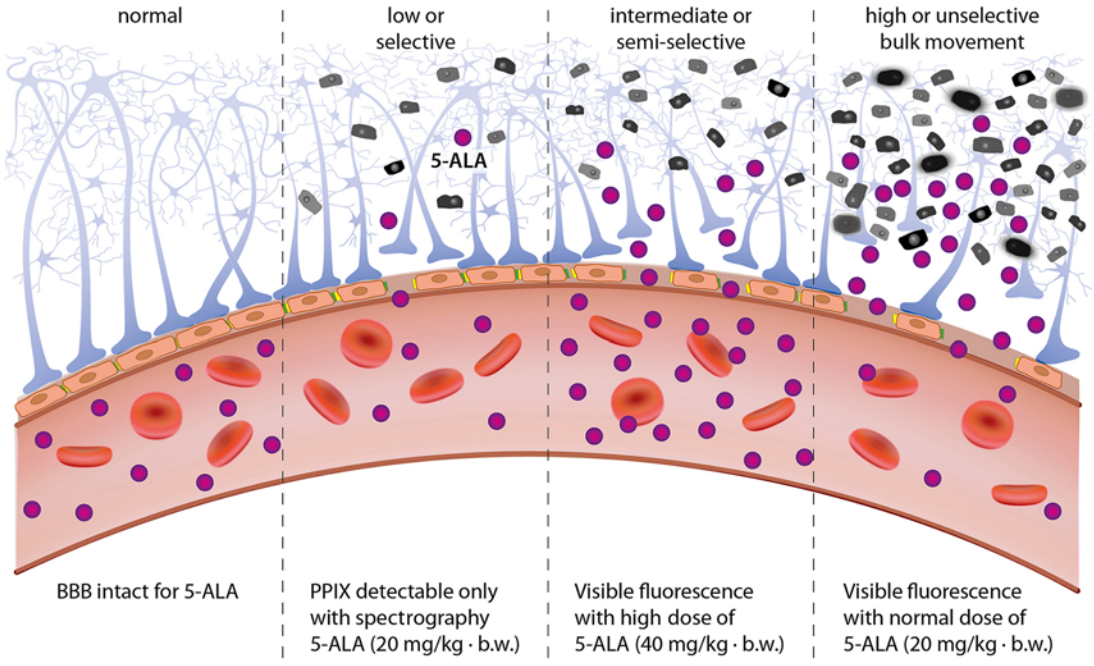

FIG. 5. Scheme illustrating the presented hypothesis of different permeability status of the BBB in glioma. Although the BBB in normal brain tissue remains intact for 5-ALA, LGGs often demonstrate a low permeability for a small amount of 5-ALA, creating fluorescence that is not visible for the human eye and only measured by spectrography. In an intermediate or semiselective BBB, a higher 5-ALA dose facilitates its uptake and creates visible fluorescence during surgery. In malignant glioma with highly disrupted BBB, the unselective bulk movement of 5-ALA through the BBB and thus the PPIX accumulation is not limited by supply but rather by the tumor's own capacity for accumulating and metabolizing porphyrins. Figure is available in color online only.

5-ALA plasma concentration. Finally, it is feasible to believe that in malignant glioma regions, where the BBB is highly disrupted, PPIX accumulation would be restricted not so much by supply, but rather by the cell capacity for accumulating and metabolizing porphyrins.

Future Thoughts

After administering a double dose (40 mg/kg bw) instead of a single dose (20 mg/kg bw) of 5-ALA 3–4 hours prior to surgery, we observed in patients with LGG a 95% increase of individuals who exhibit intraoperative fluorescence (not significant), a 138% increase in the mean cPPIX of weakly fluorescing tumors, a 90% increase of the percentage of tumors with low to medium cPPIX ($p < 0.001$), and a 300% increase in the overall mean cPPIX ($p < 0.001$). It seems that a double dose of 5-ALA on average doubles fluorescence in tumors. Tentatively, this would imply a linear relationship between dosage and fluorescence. It does not seem to affect tumors with high BBB permeability that would otherwise have had an extremely high concentration anyway, but it does increase the frequency of low to medium concentrations in the 0.5–12 μg/ml range. In the infiltrating zone or in marginal tumor boundaries of HGG, selective permeability may impair crossing of 5-ALA into the brain interstitial space. Thus, uptake might be directly influenced by the amount of 5-ALA in plasma. Consequently, in HGG, a higher (double) 5-ALA dose might be of value to better detect tumor tissue in the infiltration zone. In a recent study from our group, we observed that weak fluorescence in tumors peaked later than strong fluorescence.[24] A combination of a time-dependent analysis in relation to the administered dose could therefore be of interest in HGG as well.

Even though the amount of data available is limited, in this article we present promising results, with double-dose administration of 5-ALA offering an auspicious outlook for increasing visible and measured fluorescence in LGG.

Limitations

The small sample size of this cohort is a limitation that merits mention. Therefore, this study might be considered as underpowered. However, 5-ALA administration is off-label use in LGG. Compassionate use of 5-ALA according to published parameters as mentioned in this study can be considered. Given that we included previous tumors that could potentially be HGGs according to preoperative imaging, age of the patient, or finding in PET, patients in this cohort were initially not intended to be part of a purely LGG study. A more systematic evaluation of dosage and clinical efficacy of 5-ALA in LGG in a prospective randomized controlled setting should therefore be performed.

Conclusions

A single (20 mg/kg bw) versus double (40 mg/kg bw) dose of 5-ALA does not appear to increase the incidence of high cPPIX or strongly fluorescent tumor areas. However, it increases the cPPIX in tumor regions where cPPIX

is below the visibility threshold with single doses. Our findings indicate the role of permeability in the BBB for 5-ALA uptake into LGG which, in selected tumors, can be increased with higher 5-ALA doses.

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Conception and design: Suero Molina, Stummer. Acquisition of data: Suero Molina, Kaneko. Analysis and interpretation of data: Suero Molina, Black, Stummer. Drafting the article: Suero Molina, Black, Stummer. Critically revising the article: Suero Molina, Black, Müther, Stummer. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Suero Molina. Statistical analysis: Suero Molina, Black. Administrative/technical/material support: Suero Molina, Kaneko, Müther, Stummer. Study supervision: Suero Molina, Stummer.

Supplemental Information

Previous Presentations

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