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ORIGINAL ARTICLE - PEDIATRIC NEUROSURGERY

Spectroscopic measurement of 5-ALA-induced intracellular protoporphyrin IX in pediatric brain tumors

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Abstract

Objective 5-Aminolevulinic acid (5-ALA)–guided resection of gliomas in adults enables better delineation between tumor and normal brain, allowing improved resection and improved patients' outcome. Recently, several reports were published regarding 5-ALA for resection of pediatric brain tumors. The aim of the study was to determine the intracellular fluorescence of protoporphyrin IX (PPIX) in pediatric brain tumors by hyperspectral imaging and to compare it with visually observed intraoperative fluorescence.

Methods 5-ALA was administered orally 4 h prior to surgery. During tumor resection, the surgeon assessed the fluorescence signal to be strong, weak, or absent. Subsequently, fluorescence intensity of tumor samples was measured via spectroscopy. In addition, clinical data, imaging, and laboratory data were analyzed.

Results Eleven children (1–16 years) were operated. Tumor entities included three ($n = 3$) medulloblastomas, two ($n = 2$) pilocytic astrocytomas (PA), two ($n = 2$) anaplastic ependymomas and one ($n = 1$) diffuse astrocytoma, anaplastic astrocytoma ($n = 1$), pilomyxoid astrocytoma ($n = 1$) and anaplastic pleomorphic xanthoastrocytoma ($n = 1$). Strong fluorescence was visible in all anaplastic tumors and one PA; one PA demonstrated weak fluorescence. Visible fluorescence was strongly associated with intracellular fluorescence intensity and PPIX concentration ($P < 0.05$). Within all tumors with visible fluorescence, the intracellular PPIX concentration was greater than 4 μg/ml. Except for moderate and transient elevation of liver enzymes, no 5-ALA related adverse events were reported.

Conclusion We demonstrate a strong association between intraoperative observations and spectrometric measurements of PPIX fluorescence in tumor tissue. As in former studies, fluorescence signal was more commonly observed in malignant glial tumors. Further prospective controlled trials should be conducted to investigate the feasibility of 5-ALA-guided resection of pediatric brain tumors.

Keywords 5-ALA · PPIX · Pediatric brain tumors · Tumor resection

Introduction

Brain tumors are the most common solid malignant tumors in children and adolescents and cause the highest mortality from malignant disease [7, 9, 21]. Surgical resection of these tumors is usually the first and most important treatment method in a multimodal therapy algorithm. The main goal of surgery is to remove the tumor as wide as possible without causing neurological deficits [2, 7, 9, 13, 15–17, 21, 23, 35, 36, 41].

5-Aminolevulinic acid (5-ALA) is a metabolite of heme biosynthesis that triggers accumulation of fluorescent porphyrins, especially protoporphyrin IX (PPIX), in several brain tumor entities [20, 30]. 5-ALA-guided resection of malignant gliomas in adults increases the rate of complete resection without causing additional neurological deficits [31]. Higher rates of complete resections are associated with better progression-free survival (PFS) and overall survival (OS), as demonstrated in numerous studies [6, 18, 25, 31, 32, 37].

Michael Schwake, Sadahiro Kaneko, Angela Brentrup and Walter Stummer contributed equally to this work.

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In vitro experiments [26, 27], case reports, and case series [3, 4, 8, 19, 22, 24, 29, 33] suggest that 5-ALA-guided resection could be of use in the resection of brain tumors in children and adolescents. 5-ALA-guided resections of pediatric brain tumors are performed off-label with the necessary informed consent in our department. In order to objectively determine PPIX concentration, resected tumor samples underwent ex vivo spectrometric measurements. We collected these data to better understand the capacity of various pediatric brain tumor types to accumulate PpIX after oral administration of 5-ALA considering a possible use for fluorescence-guided resection and/or photodynamic therapy (PDT).

Materials and methods

5-ALA-guided resection of pediatric brain tumors is performed in our department on an off-label basis with patients and guardians giving full informed consent prior to surgery. For this purpose, Gliolan® (medac, Wedel, Germany) was administered orally 4 h prior to surgery. The surgical procedure did not differ from the standard procedure for resection of brain tumors in patients under 18 years of age in our department. After induction of anesthesia, patients were placed either in a supine position, for tumors in the temporal, parietal, or frontal lobe, or in a "park bench" position for tumors in the occipital lobe or posterior fossa. After positioning, a skin incision and craniotomy were performed, adapted to the location of the tumor, followed by opening of the dura. Tumor resection was performed using microsurgical instruments as well as an ultrasound aspirator (CUSA clarity, Integra, Ratingen, Germany). The resection was performed with the help of a surgical microscope (Pentero, Carl Zeiss, Oberkochen, Germany) with the BLUE400 filter option. During tumor resection, we switched to the BLUE400 filter for identifying fluorescing tissue. The goal of all operations was, when feasible, a complete resection. Finally, watertight closure of the dura mater, cranioplasty, and wound closure was performed. A postoperative MRI was performed within 48 h. Depending on the location of the tumor, neuro-navigation, intraoperative monitoring, and intraoperative ultrasound were furthermore utilized. All operations were conducted by one of the senior authors AB or WS.

In addition, between February 2018 and January 2019, spectrometric measurements were conducted on tissue samples from tumors operated after the administration of 5-ALA. All procedures were conducted in accordance with the ethical standards of the local ethical committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Spectrometric measurement

Spectrometric measurements were performed as described previously [11, 38, 40]. Briefly, fluorescence intensity was measured using a continuous hyperspectral imaging system. A light-emitting diode (LED) was coupled into OPMI pico (Carl Zeiss Meditec, Oberkochen, Germany) via a liquid light guide. A filter swivel joint to a BLUE 400 observation filter was installed in order to eventually record BLUE 400 images. Individual components camera, LED light source, filters, and spectrometer were controlled by LabVIEW (National Instruments, Inc.; Austin, TX) software. Images in fluorescent mode, dark images, and white light images were taken in succession. Fluorescence spectra and white light spectra were recorded for the entire wavelength range of the filter from 420 to 730 nm. Measurements were started at the longest wavelength and minimized in 3 nm steps for the fluorescence spectra and in 5 nm for the white light spectra of the wavelength range of the filter.

The fluorescence intensities were measured ex vivo in 10 regions of interest (ROI) per tumor sample biopsy. The data was corrected for short-term fluctuations in excitation light by a reference that was measured before each tissue measurement. Reference measurements were made with a non-bleaching reference object (635 nm fluorescence phantom as supplied with microscope, Carl Zeiss). The values were averaged to obtain final fluorescence intensity for each ROI. In order to calculate PPIX concentrations, the fluorescence spectra were first normalized by reference values to correct for inhomogeneous tissue absorption and scattering characteristics. Tissue PPIX concentrations were calculated using MATLAB (The Math Works, Inc., Natick, MA) also as described previously, using an algorithm made from measurements of phantoms with known PPIX concentrations [11].

Analysis of the postoperative course

In addition, clinical data including neurological status, as routinely obtained, and surgical complications were recorded. Postoperative MRI images were evaluated and compared with the preoperative images. Blood count, kidney, and liver parameters were examined in all patients before surgery, at the day after surgery, and before discharge, as part of standard therapy protocol.

Statistics

All data analyses were performed with commercially available software (Statistical Package for Social Sciences, Version 23.0; SPSS; Inc., Chicago, IL). Data were described by standard descriptive statistics. We used two-tailed student's t tests for metrical, normally distributed variables, Mann-Whitney U tests (MWU) for ordinal or metrical not normally distributed variables, and Fisher's exact test for categorical and dichotomous variables, as appropriate. Statistical tests were considered significant at probability level of $P < 0.05$.

Results

5-ALA-guided resection and intraoperative fluorescence

Eleven children aged between one and 16 years were operated (female $N = 6$, male $N = 5$). Tumor entities included medulloblastoma WHO grade IV ($N = 3$, two of them without sonic hedgehog (SHH) or wnt pathways (WNT) activation, the third group (3), pilocytic astrocytoma WHO grade I ($N = 2$), anaplastic ependymoma WHO grade III ($N = 2$), diffuse astrocytoma WHO grade II ($N = 1$), anaplastic astrocytoma WHO grade III ($N = 1$), pilomyxoid astrocytoma (PA) WHO grade II ($N = 1$), and anaplastic pleomorphic xanthoastrocytoma WHO grad III ($N = 1$). Strong fluorescence signals were observed in all anaplastic tumors and in one PA, whereas one PA demonstrated weak fluorescence signal. No fluorescence was detected in any of the analyzed medulloblastomas, in diffuse astrocytoma WHO grade II and pilomyxoid astrocytoma (Table 1). Further analyses regarding association between fluorescence signal and tumor type and extent of resection were not possible due to the small number of patients.

Spectrometric measurement

From 11 tumors, we conducted spectrometric measurements in a total of 28 tissue samples. These measurements showed a significantly higher fluorescence intensity and PPIX concentration in the samples from tumors with strong fluorescence in comparison to those tumors with absent or weak fluorescence ($P < 0.05$). In addition, we determined a higher fluorescence intensity and PPIX concentration in the samples from tumors with weak fluorescence in comparison to tumors with no fluorescence signal at all ($P < 0.05$) (Fig. 1). Moreover, we found a clear cutoff for the required concentration of PPIX in tumor tissue for the intraoperative visualization of PPIX fluorescence signal. In all tumors with visible fluorescence, the PPIX concentration exceeded 4 μg/ml. In contrast, PPIX concentrations in tumors without visible fluorescence remained below 0.20 μg/ml (Fig. 2).

Postoperative course

Laboratory data showed a moderate elevation of the liver enzymes alanine aminotransferase (ALT) (median 26 U/l, IQR 22–33.5) and aspartate aminotransferase (AST) (median 38 U/l, IQR 34–52.5) in comparison to preoperative values (ALT: median 16 U/l, IQR 12–18.5; AST: median 22 U/l, IQR 19.5–26) ($P < 0.05$). However, both median levels remained within reference levels (ALT < 40 U/l, AST < 45 U/l) (Fig. 3). One of the children had a ventriculitis after ventriculostomy, which was not associated with the use of 5-ALA and was treated by antibiotics; no further 5-ALA-adverse events were reported; neither anaphylactic reactions, photosensitivity, nor new postoperative neurological deficits were observed (Table 1).

Discussion

Spectrometric measurements of tumor samples show a high association between the intraoperative findings and the spectrometric determined fluorescence intensity and calculated concentration of PPIX. Furthermore, this small cohort study showed no undesired effects of 5-ALA apart from the known clinical irrelevant slight increase of liver transaminases ALT and AST in pediatric patients [4, 31].

Table 1 Demography of children and adolescents with brain tumors operated between February and November 2018. *GTR* gross total resection, *STR* subtotal resection

Sex	Age (years)	Histology	WHO grade	Primary/ recurrence	Intraoperative fluorescence	Extent of resection	Complication
Female	11	Pilomyxoid astrocytoma	II	Primary	No signal	GTR	None
Male	16	Diffuse astrocytoma	II	Primary	No signal	STR	None
Female	16	Pilocytic astrocytoma	I	Recurrent	Strong	GTR	None
Female	3	Anaplastic Astrocytoma	III	Recurrent	Strong	GTR	None
Male	13	Anaplastic pleomorphic xanthoastrocytoma	III	Primary	Strong	GTR	None
Female	5	Medulloblastoma	IV	Primary	No signal	GTR	None
Male	3	Medulloblastoma	IV	Primary	No signal	GTR	Ventriculitis
Female	1	Anaplastic ependymoma	III	Recurrent	Strong	STR	None
Male	10	Anaplastic ependymoma	III	Primary	Strong	GTR	None
Male	9	Pilocytic astrocytoma	I	Primary	Weak	GTR	None
Female	10	Medulloblastoma	IV	Primary	No signal	STR	None

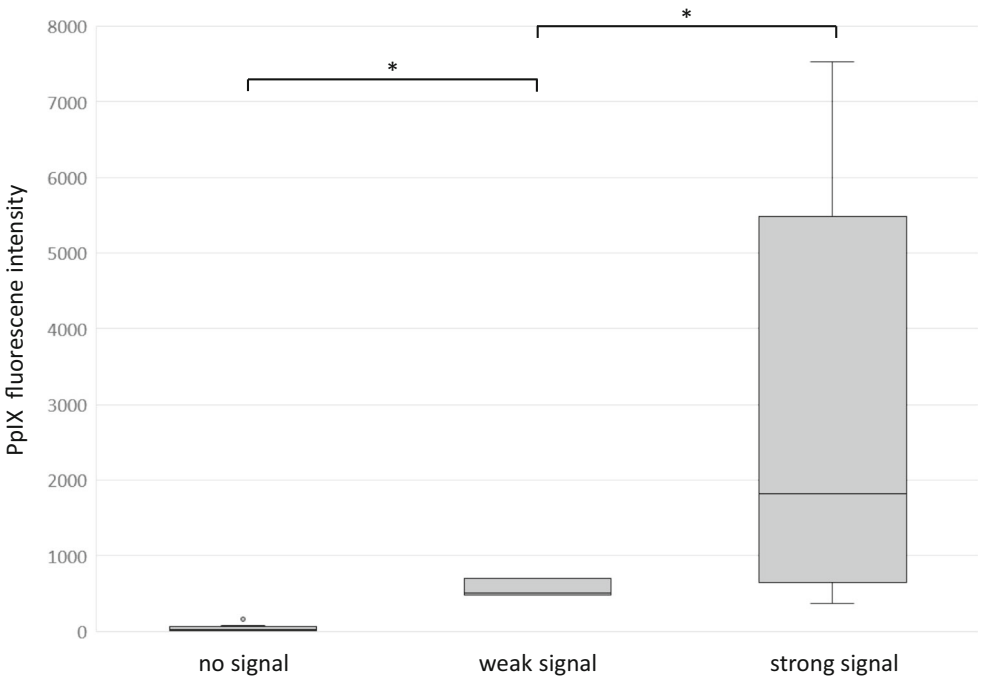

Fig. 1 Box plot diagram shows the measured fluorescence intensity in the various tumor samples. No signal: tumors without fluorescence intraoperatively; weak signal: tumors with weak fluorescence; and strong signal: tumors with strong fluorescence. Asterisk marks statistical significance ($P < 0.05$)

5-ALA-guided tumor resection

The important role of maximal resection of brain tumors in children and adolescents is well acknowledged. A complete resection is extremely important to enable improvement of prognosis with longer PFS and OS [1, 7, 13, 15, 21, 23, 35, 36, 41]. At the same time, the resection must be safe; not causing additional neurological deficits result due to an injury of surrounding functional brain areas is paramount. Many methods have been established in recent years to help surgeons with this task. One of these is 5-ALA-guided resection. Many studies on adults have shown that 5-ALA-guided resection leads to a significant higher rate of GTR of malignant gliomas, and thus better prognosis with prolonged PFS and

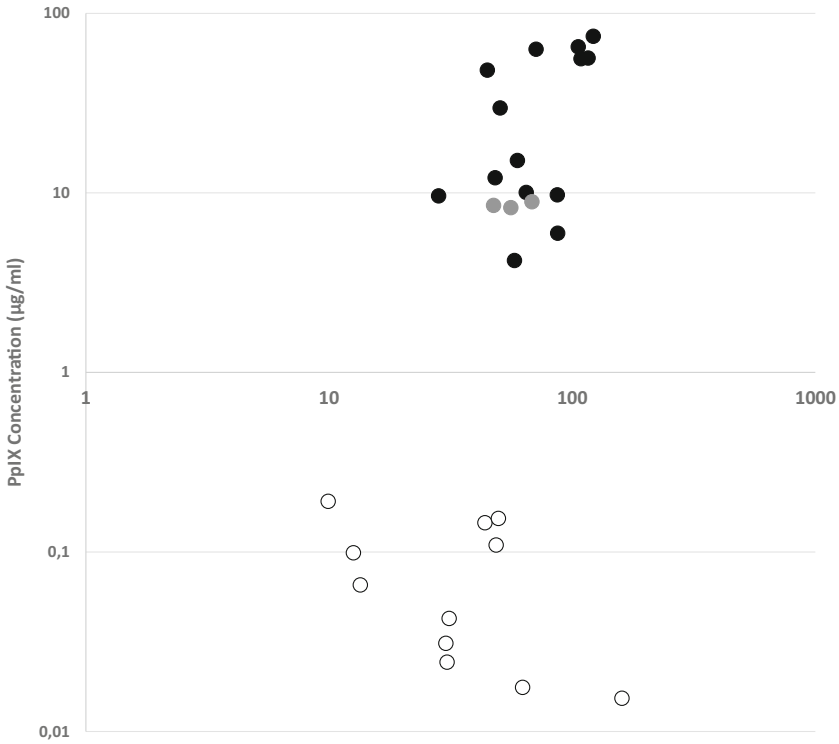

Fig. 2 A logarithmic scatter diagram demonstrating the relation between the calculated PPIX concentration in tumor tissue samples and the intraoperative findings. White dots: no signal; black dots: strong signal; gray dots: weak signal. This diagram shows the clear cutoff of the required PpIX concentration in tumor tissue to enable intraoperative visualization of the fluorescence signal. All tumors with visible fluorescence signal had a PPIX content greater than 4 µg/ml

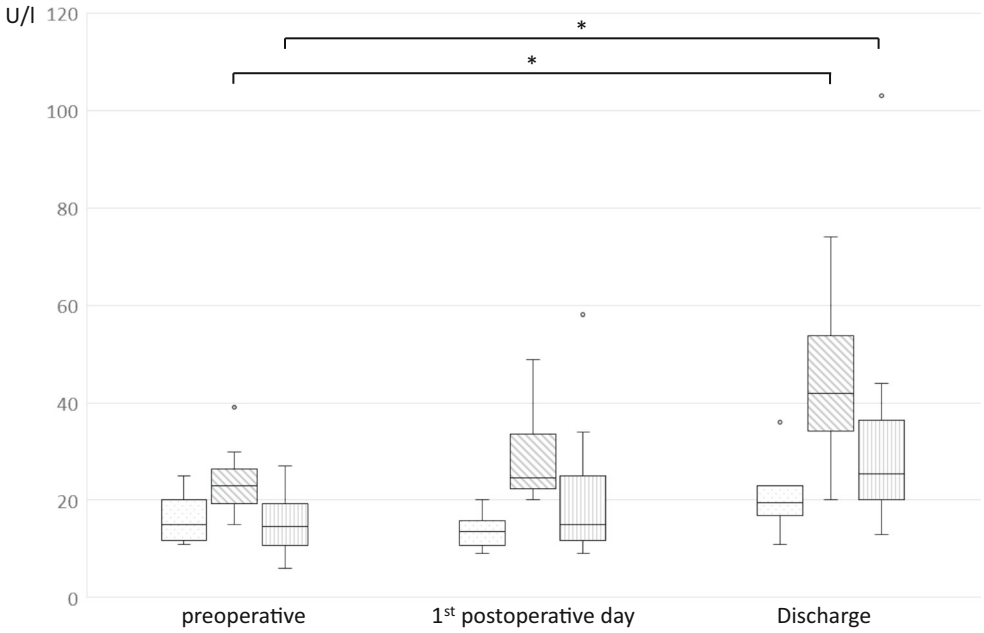

Fig. 3 Box plot diagram shows the change in liver enzymes after the administration of 5-ALA (Gliolan®). There is a significant increase in aspartate aminotransferase (AST, oblique gray lines) and alanine aminotrasferase (ALT, straight gray lines) ($P < 0.05$, marked with asterisk). No significant elevation was observed in gamma-glutamyltransferase (γGT, gray dots). However, median values of ALT and AST remained below the normal values (normal values: γGT 5–17 U/l, ALT < 40 U/l, AST < 45 U/l)

OS [6, 18, 25, 32, 37]. Due to the selective accumulation of PPIX in the tumor cells [5, 10, 34, 38, 39], the surgeon is able to detect the tumor margins easily during surgery, thus resecting the tumor tissue while keeping the surrounding brain tissue intact. In fact, no higher rate of complications was reported in 5-ALA-guided resections despite the higher amount of resections, especially not a higher rate of postoperative neurological deficits [31].

Potential role of 5-ALA in pediatric brain tumors

Studies on cell cultures of typical brain tumors of children and adolescents from our group showed that incubation of the cells with 5-ALA leads to an accumulation of fluorescent PPIX [26]. This suggests that such a method might also be efficient in vivo. However, clinical experience shows that PpIX fluorescence is very variable and depends on the tumor type. A strong and useful fluorescence had been observed mainly in malignant astrocytomas and ependymomas; however, in the more frequent medulloblastomas and pilocytic astrocytomas, fluorescence signal was not uniform and often not useful for the surgeon [3, 4, 8, 19, 22, 24, 28, 29, 33]. According to the current WHO classification, there are numerous types and subtypes of brain tumors in children and adolescents. Compared to adults, in whom nearly 90% of CNS tumors are astrocytomas, metastases, or meningiomas; the distribution under the age of 18 years is significantly more variable [9, 14]. Astrocytomas and embryonic tumors are the most common, followed by ependymomas. Since there is a very high number of different tumor types in children and adolescents compared to adults, and since the overall incidence is very low [9], it is impossible to answer all questions in a small case series. Therefore, a general recommendation for 5-ALA-guided surgery cannot be given at this time. On the other hand, an analysis of 175 published cases of 5-ALA-guided surgery on brain tumors in children and adolescents showed that 5-ALA-guided resection had an influence on the grade of resection ($P < 0.001$) [28]. The published results indicate that the utility of 5-ALA-guided resection might play a role in the resection of brain tumors of children and adolescents, particularly in contrast-enhancing, supratentorial tumors [33].

The aim of this study was to compare the intraoperative finding to spectrometric measurements of the intracellular fluorescence and PPIX concentration in tumor tissue. Importantly, we detected a clear cutoff of a minimum PPIX concentration necessary for allowing visual recognition of the fluorescence signal using the adapted surgical microscope. The results indeed showed a strong association between the spectrometric and intraoperative findings. In accordance with previous case reports and series, we observed and detected strong fluorescence signals and higher PPIX concentrations in the four malignant astrocytomas (grade III according to WHO, $N = 4$, 100%). No fluorescence was found in the two grade II astrocytomas ($N = 2$, 0%). In addition, no fluorescence signal was detected in all three medulloblastomas ($N = 3$, 0%). However, fluorescence was observed in the two pilocytic astrocytomas ($N = 2$, 0%). Lastly, it should also be mentioned that we did not detect any side effects related to 5-ALA, with the exception of a slight increase in liver transaminases [4, 31].

Limitations

Due to the small number of patients with different tumor entities, it was impossible to determine any influence of 5-ALA-

guided resection on grade of resection. Moreover, the clinical data allocated in this study, except spectrometric measurements, were analyzed retrospectively.

Conclusion

We showed that intraoperative observations and spectrometric measurements of fluorescence intensity and PPIX concentration in tumor tissue are closely related. As in previous studies, the fluorescence signal was more frequent and stronger in malignant glial tumors. No major adverse events associated with 5-ALA were observed. Furthermore, interventions aiming at increasing tumor PPIX [12] will have to be tested using objective methods of PPIX measurements, such as spectrography. To investigate the role of 5-ALA-guided resection of pediatric brain tumor, prospective controlled trails are required.

Compliance with ethical standards

Conflict of interest Walter Stummer has received consultant fees from medac, (Wedel, Germany), Carl Zeiss Meditech (Oberkochen, Germany), and NxDc (Lexington, KY, USA). All other authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional ethical committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (2019-400-f-S).

Informed consent Informed consent was obtained from all individual participants included in the study.

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