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Research Article

Quantitative Analysis of 5-aminolevulinic Acid Fluorescence Intensity in Glioma Surgery: A Retrospective Study

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Abstract

Objective: 5-aminolevulinic acid (5-ALA) is used for intraoperative tumor visualization in neurosurgery. We hypothesized that 5-ALA fluorescence intensity may vary according to glioma pathology.

Methods: We investigated 28 patients with glioma who underwent surgery between 2019 and 2023. 5-ALA was administered before surgery, and the intensity of 5-ALA fluorescence was quantified intraoperatively. The pathological diagnosis was based on the 2016 World Health Organization classification.

Results: Seventeen patients with glioblastoma (GBM), four with anaplastic astrocytoma (AA), three with oligodendroglioma (OL), and four with low-grade glioma (LGG) were examined. The mean (SD) intensity of the 5-ALA fluorescence was 155.9 (56.0) in GBM, 180.5 (64.7) in AA, and 66.3 (18.8) in OL. The intensity in OL was significantly lower than that in GBM and AA (Scheffé's test, $P < .05$). All LGGs were negative.

Conclusions: The intensity of 5-ALA fluorescence varies by pathology, which may contribute to intraoperative diagnostic support.

Keywords: 5-Aminolevulinic Acid (5-ALA); Anaplastic Astrocytoma (AA); Magnetic Resonance Imaging (MRI)

Introduction

Despite recent advances in diagnosis and treatment methods, the prognosis of malignant gliomas has not shown significant improvement. Although the tumor removal rate correlates with prognosis, malignant glioma which has invasive growth characteristics is difficult to distinguish from normal tissue, even if the boundaries appear distinct in gadolinium-enhanced magnetic resonance imaging (MRI). Therefore, intraoperative fluorescence diagnosis using 5-aminolevulinic acid (5-ALA) has been adopted to improve selective tumor removal.

Fluorescence-guided surgery using 5-ALA is used to clarify the boundary between the tumor and normal brain tissue and to achieve complete resection of malignant gliomas [1]. According to Stummer's classification, 5-ALA positive findings are classified into three levels: strong, vague, and none; however, this is an observer's visual assessment and is not quantitative [2,3]. Therefore, we quantified the intensity of 5-ALA fluorescence using open-source imaging software (ImageJ, National Institutes of Health, Bethesda, MD, USA) [4,5]. We hypothesized that glioma pathology may vary according to the intensity of 5-ALA fluorescence and examined it in this study.

Methods

Patients

This retrospective study included 28 patients with gliomas who underwent removal surgery using intraoperative 5-ALA fluorescence between 2019 and 2023 in the Department of Neurosurgery at Showa Medical University. These 28 patients were selected according to the following three criteria: 1) tumor removal surgeries were performed for the first time; 2) the patients agreed to use 5-ALA; and 3) postoperative pathology showed clear results of glioma. The pathological diagnosis was based on the 2016 World Health Organization classification. 5-ALA was administered before surgery, and the intensity of 5-ALA fluorescence was quantified. This retrospective study was approved by the Research Ethics Board of Showa Medical University (#21-074B). Informed consent was obtained from the patients and their families before surgery.

Quantitative analysis of 5-ALA fluorescence intensity

In each patient, 20 mg/kg aminolevulinic acid hydrochloride (Nobelpharma Co., Ltd.) was orally administered 2 h before entering the operating room. Intraoperatively, we used a fluorescence microscope (Leica OH4, Leica Co., Wetzlar, Germany) and Esperaluz type 2 (CCS Co., Ltd.) as luminescence devices. Using these instruments, 5-ALA fluorescence imaging was performed on resected tumor specimens. The clearest image of the samples from each patient was selected for quantification. To avoid inter-observer discrepancy, the procedures were performed with the same conditions, light intensity, and surgeons in all patients.

Quantitative intensity analysis of 5-ALA fluorescence using ImageJ was conducted as follows: 1) the 5-ALA fluorescence image was converted to an image file in 8-bit format; 2) the file was imported as image stacks; 3) the region of interest was set at the site of the tumor; and 4) the maximal intensity of the entire image was calculated. All procedures were performed according to patient data.

Statistical analysis

We compared the maximal intensity of 5-ALA fluorescence with the postoperative pathology results using Scheffé's test. Statistical analyses were performed using Microsoft Excel.

Results

The patient characteristics are summarized in Table 1. Seventeen cases of glioblastoma (GBM), four cases of anaplastic astrocytoma (AA), three cases of oligodendroglioma (OL), and four cases of low-grade glioma (LGG) were examined. The mean (SD) intensity of 5-ALA fluorescence was 155.9 (56.0) in GBM, 180.5 (64.7) in AA, and 66.3 (18.8) in OL. Brightness in the OL was significantly lower than that in the GBM and AA groups (Scheffé's test, $P < .05$, Figure 1). All LGGs were negative. The results of quantitative intensity analysis of 5-ALA fluorescence in a representative case of GBM are shown in Figure 2.

Patient characteristics	
Gender, n (%)	
Female	13 (46.4)
Male	15 (53.6)
Age, mean (SD), years	56.3 (17.0)
Pathology, n (%)	
GBM	17 (60.7)
AA	4 (14.3)
OL	3 (10.7)
LGG	4 (14.3)
Lesion side, n (%)	
Right	11 (39.3)
Left	17 (60.7)
Lesion location, n (%)	
Frontal	14 (50.0)
Temporal	5 (18.0)
Parietal	2 (7.1)
Central	1 (3.6)
Occipital	2 (7.1)
Tempora-occipital	2 (7.1)
Parieto-occipital	2 (7.1)
MRI boundry, n (%)	
Sharp	9 (32.1)
Unclear	19 (67.9)

Table 1

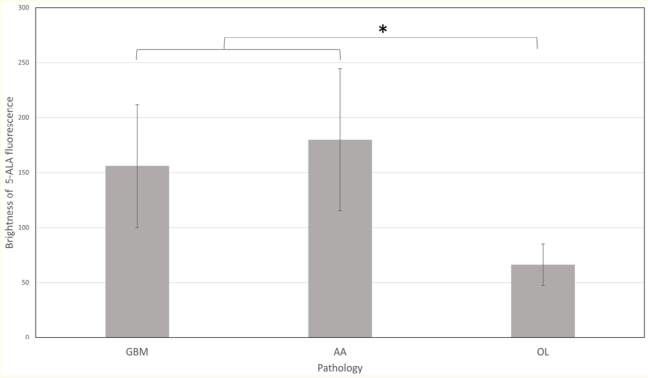

Figure 1: Scheffé's test revealed significant differences in the intensity of 5-ALA fluorescence among oligodendroglioma (OL), glioblastoma (GBM), and astrocytoma (AA). *, P < 0.05.

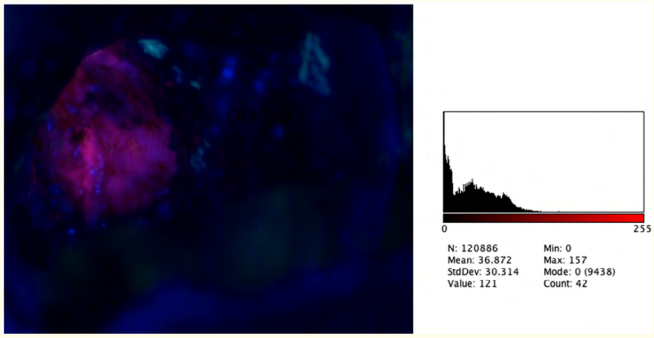

Figure 2: A case of glioblastoma. The tumor was clearly 5-ALA fluorescence positive. Quantitative intensity analysis of 5-ALA fluorescence showed maximum value of 157.

Discussion

This is the first report to show that the intensity of 5-ALA fluorescence tends to vary with glioma pathology. The relationship between 5-ALA luminescence and molecular pathology remains unclear [6]. Ohba., *et al*. reported that mutant isocitrate dehydrogenase (IDH) indirectly reduces the amount of exogenous 5-ALA-derived protoporphyrinogen IX (PpIX) in glioma cells [7]. Yoneda., *et al*. also demonstrated that 5-ALA-induced PpIX fluorescence quantitatively correlates with histopathological malignant features in strongly and vaguely fluorescent areas [8]. Our results are consistent with their report. In contrast, Maria., *et al*. [9] reported no positive relationship between molecular status and 5-ALA. Photodynamic diagnosis is a useful tool for the supratotal removal of brain tumors [2] and is useful for tumors other than gliomas; positive findings were reported for meningioma, lymphoma, and metastatic brain tumors [10]. However, there are many unclarified aspects regarding the relationship between various tumor pathologies and 5-ALA fluorescence intensity and we further investigated this issue, including performing an intraoperative analysis (Figure 3).

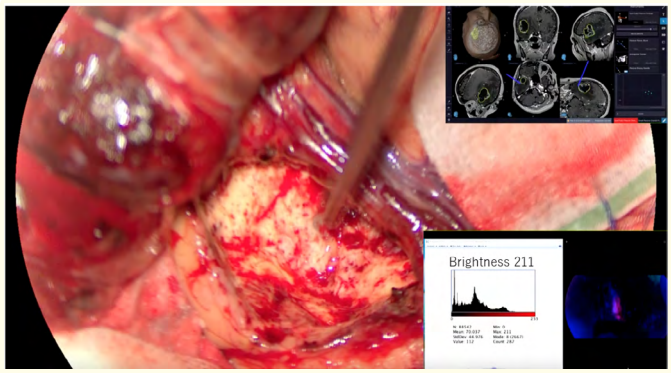

Figure 3: Intraoperative quantitative analysis of 5-ALA fluorescence intensity in a case of right temporal glioblastoma. The strongly positive 5-ALA labeled region was submitted for rapid histopathological diagnosis. The maximum intensity was 211, which supported the histopathological diagnosis of glioblastoma.

In this study, all four LGG cases were negative for 5-ALA fluorescence. Jaber., *et al*. [11] reported that sixteen of seventy-four LGGs (21.6%) fluoresced in their study. As they pointed out, 5-ALA fluorescence appears to be an inherent marker of malignant transformation and overall survival; hence, regions with 5-ALA fluorescence should be aggressively considered even in cases where LGG is suspected preoperatively.

Although Stummer's classification is defined as strong, vague, or none based on visual observation, assessments vary by investigator and are sometimes difficult to accurately classify [3]. The ventricle walls are known to be positive for 5-ALA fluorescence and their intensity is lower than that of the tumors, indicating that they may be closer to Stummer's classification of "vague". In cases such as intraventricular tumors, it is necessary to precisely determine the boundary between the ventricles and tumor by intraoperative quantitative evaluation of 5-ALA fluorescence.

The limitations of this study include biases in the assessment of the fluorescence intensity, surgeon's visual acuity, surgical field conditions, such as distance to the optical axis, the relationship with the light intensity, and the effects of preoperative drug administration (such as corticosteroids and antiepileptic drugs). In cases of deep tissue using an endoscope, it is currently thought that light can only be sensed at a straight-line distance, owing to the angle of the optical axis. To overcome this problem, a device that can use 5-ALA in combination with an endoscope or exoscope was developed [12]. Another limitation is the difference in the intensity of the light sources [13]. Optimal access and evaluation pitfalls should be considered.

Conclusion

The intensity of 5-ALA fluorescence varies by glioma pathology. Quantitative analysis of 5-ALA fluorescence intensity may aid intraoperative diagnostic assessment during glioma surgery.

Conflicts of Interest and Source of Funding

Funding information is not available. The authors of this work have nothing to disclose.

Data Availability Statement

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

Author Contributions

Takashi Kon: Methodology, Formal analysis, Writing – original draft. Yosuke Sato: Conceptualization, Supervision, Investigation, Writing - review and editing. Yusuke Kobayashi: Investigation. Kosuke Tanaka: Investigation. Junichiro Takahashi: Investigation. Yoichi Morofuji: Investigation.

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