Histological Examination of False Positive Tissue Resection Using 5-Aminolevulinic Acid-Induced Fluorescence Guidance

Satoshi UTSUKI, Hidehiro OKA, Sumito SATO, Satoru SHIMIZU, Sachio SUZUKI, Yoshinori TANIZAKI, Koji KONDO, Yoshiteru MIYAJIMA, and Kiyotaka FUJII

Department of Neurosurgery, Kitasato University School of Medicine, Sagamihara, Kanagawa

Abstract

Intraoperative 5-aminolevulinic acid (5-ALA)-induced fluorescence guidance for resection of malignant brain tumors was correlated with histological examination to investigate false positive findings in 42 patients with malignant glioma and six patients with metastatic brain tumor. Patients received a single 1 g oral dose of 5-ALA 2 hours before surgery. The tumor site was illuminated with a laser with a peak wavelength of 405 ± 1 nm and output of 40 mW. Samples with strong fluorescence were obtained from the tumor bulk and samples with weak fluorescence from the tumor cavity. Fluorescence was observed in 36 of the 42 malignant gliomas and four of the six metastatic brain tumors. No tumor cells were found in fluorescent samples from six of the 36 malignant gliomas and all four metastatic brain tumors. Five of the six malignant gliomas were recurrent cases. Fluorescence was found in areas of peritumoral edema or inflammatory cell and reactive astrocyte infiltration. Intraoperative 5-ALA-induced fluorescence guidance is useful for the resection of initial malignant glioma since false positive results are rare, but only non-eloquent weak positive areas should be resected. In contrast, all weak positive areas of recurrent malignant gliomas must be resected. Weak positive areas of the peritumoral edema surrounding metastatic brain tumors should be removed carefully as false positive results are common.

Key words: 5-aminolevulinic acid, false positive, fluorescence diagnosis, macrophage, reactive astrocyte

Introduction

The heme precursor 5-aminolevulinic acid (5-ALA) is an amino acid derivative that is metabolized by mitochondria via heme-biosynthesis pathway enzymes into the fluorescent molecule, protoporphyrin IX (PPIX).5) 5-ALA given via oral administration normally does not enter the normal brain tissue because of the hydrophobic character of the brain and the presence of the blood-brain barrier (BBB).17) However, 5-ALA is taken up in brain tumor cells, forming excess PPIX, because of the damaged BBB in brain tumor.23) The PPIX density also leaks into the edematous tissue surrounding the tumor through the damaged BBB.13,19) Exposure to an appropriate wavelength of light causes PPIX to fluoresce,8) allowing intraoperative diagnosis of neoplastic cells by visual differentiation from surrounding normal cells.6,18,20,21) Intraoperative fluorescence-guided malignant brain tumor resection can use PPIX fluorescence for chemonavigation to achieve total extirpation of the tumor. Other dyes, such as photofrin15) and fluorescein Na,10) may be carried into the surgical cavity by blood, thus threatening the guidance selectivity. In contrast, 5-ALA must first be converted into the fluorescent PPIX by the tumor cell. The sensitivity of 5-ALA-induced fluorescence is 82% for bronchial cancer,14) 94% for bladder cancer,20) and 92% for ovarian carcinoma metastases.11) However, inflammatory reactions in bacterial, chemical, or irradiated scar tissue may cause false positive findings. In particular, strong inflammatory reactions seem to induce a high false positive rate.5) Therefore, chemonavigation using fluorescent tissue may result in resection of normal brain tissue.

The present study evaluated the risk of excess resection of brain tissue during bulk tumor resection using 5-ALA-induced fluorescence by histological examination of the resected fluorescent tissue.
Materials and Methods

Thirty patients with glioblastoma multiforme, including seven recurrent tumors, 12 patients with anaplastic astrocytoma, including six recurrent tumors, and six patients with six metastatic brain tumors were surgically treated at the Department of Neurosurgery of Kitasato University Hospital between 2003 and 2005. The glioblastoma multiforme and anaplastic astrocytoma cases were identified histologically according to the World Health Organization criteria. This study was reviewed and approved by the Kitasato University Hospital Ethics Committee and informed patient consent was obtained.

Two hours before the administration of anesthesia, each patient was given 1 g of 5-ALA orally. The tumor resection through a craniotomy was completed 4 to 6 hours after 5-ALA administration. The tumor site was exposed to a handheld laser (VLD-VI version 2; M & M Co., Ltd., Tokyo) with peak wavelength of 405 ± 1 nm and output of 40 mW with scanning through a fiber optic cable. Tissue was collected under the operating microscope from three to five locations within the tumor bulk with strong fluorescence, and from the surrounding area with weak fluorescence after bulk and macroscopic tumor resection. All specimens were removed by forceps, and were about 5 mm in size. All specimens were fixed with 10% formalin and embedded in paraffin. False positive was defined as the absence of tumor cells, for example, cellular polymorphism or nuclear atypia, in the fluorescent tissue.

Results

Intraoperative 5-ALA-induced fluorescence was observed in 28 of the 30 glioblastomas (6 of 7 recurrent tumors), eight of the 12 anaplastic astrocytomas (5 of 6 recurrent tumors), and four of the six metastatic brain tumors. From these 40 fluorescent tumors, 152 strong fluorescent samples and 145 weak fluorescent samples were obtained. No tumor cells were identified in two of 152 strong fluorescent samples and 25 of 145 weak fluorescent samples. Tumor cells were observed in all of the 20 strong fluorescent samples, but only two of 20 weak fluorescent samples from metastatic tumors.

No tumor cells were identified in samples from four of the 28 glioblastomas (3 of 6 recurrent tumors), two of the eight anaplastic astrocytomas (2 of 5 recurrent tumors), and all of the four metastatic brain tumors. Therefore, the rate of false positive samples was one of 25 initial malignant gliomas, five of 11 recurrent malignant gliomas, and four of four metastatic brain tumors.

The false positive fluorescence in the case of initial malignant glioma was associated with remarkable infiltration of neutrophils (Fig. 1). Fluorescence was also observed in other samples without infiltration of neutrophils. In all other cases except this one, false positive results existed in malignant gliomas. False positive fluorescence was also associated with remarkable infiltration of reactive astrocytes and macrophages in the samples of recurrent malignant gliomas (Fig. 2), and with peritumoral edema and infiltration of reactive astrocytes in the samples of metastatic brain tumors (Fig. 3).

Areas of high cellularity were seen in all strong fluorescent samples including tissue infiltrated by neutrophils, indicating that strong fluorescent samples were areas of neoplastic activity. In contrast, areas of low cellularity with edema were seen.
in all weak fluorescent samples except tissues infiltrated by reactive astrocytes and macrophages. Moreover, low cellularity with peritumoral edema and infiltration of reactive astrocytes was seen in all samples of metastatic brain tumors. Therefore, the weak fluorescent samples indicated the boundary of tumor infiltration.

**Discussion**

This study found that 5-ALA-induced fluorescence decreased in intensity further from the tumor bulk of malignant glioma. The intensity of fluorescence is inversely correlated with the tumor cellularity.\(^{10}\) Serial biopsy of malignant glioma showed that hyperintense areas on \(T_2\)-weighted magnetic resonance imaging usually correspond to infiltration of tumor cells.\(^4,7\) Even tumor bulk resection is likely to result in residual tumor cells in the operation site due to difficulty in resection of such hyperintense areas.\(^{10}\)

This study found that false positives were rare at the initial resection of malignant glioma, as 5-ALA-induced fluorescence was not observed in all hyperintense areas. The false positive was found in a case of glioblastoma with remarkable infiltration of neutrophils, which was thought to be exceptional. Therefore, the sensitivity of 5-ALA-induced fluorescence is thought to be 100% during initial resection of malignant glioma, unless inflammatory cell infiltration is present.\(^5,12\) Oncocytes are present in the majority of positive sections by 5-ALA-induced fluorescence, so surgical resection is highly recommended in non-eloquent areas and resectable parts of the initial malignant glioma.

False positive results were found in five of 11 recurrent malignant gliomas. Pseudo-positive rates were also higher in recurrent cases than initial cases. Remarkable inflammatory cell infiltration was found in the pseudo-positive specimens, indicating that PPIX or 5-ALA probably leaked through the damaged BBB and entered the inflammatory cells.\(^{12}\) 5-ALA-induced fluorescence is often observed around tumor enucleation without tumor cells due to the inflammatory cell infiltration associated with surgical and radiation intervention.\(^5\) Another important factor is that recurrent malignant gliomas must be removed.

5-ALA-induced fluorescence was observed in the edema area surrounding all metastatic tumors but without tumor cells. The major cause was probably leakage of 5-ALA or PPIX through the damaged BBB of tumor cells. Of course, tumor cells can infiltrate into the surrounding edema, but the sensitivity of fluorescence-guided resection is low because of the absence of tumor cell infiltration to all fluorescent sections in metastatic brain tumors.

5-ALA-induced fluorescence indicates areas of inflammatory cell infiltration and edema with or without tumor cells. Intraoperative 5-ALA-induced fluorescence guidance is useful for the resection of initial malignant glioma, since false positive results are rare, but only non-eloquent weak positive areas should be resected. In contrast, all weak positive areas of recurrent malignant glioma must be resected. Weak positive areas of the peritumoral edema surrounding metastatic brain tumor should be removed carefully as false positive results are common.

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**References**

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Address reprint requests to: Satoshi Utsuki, M.D., Department of Neurosurgery, Kitasato University School of Medicine, 1–15–1 Kitasato, Sagamihara, Kanagawa 228-8555, Japan.
e-mail: utsuki@med.kitasato-u.ac.jp

Commentary

Among the determinants of prognoses for malignant brain tumor, the extent of surgical resection seems to be the main factor. Therefore, methods that improve intraoperative identification of malignant tissue, enabling more selective and thorough tumor resection, may be of value. 5-ALA-induced protoporphyrin IX (PPIX) fluorescence has shown an outstanding sensitivity for the assessment of malignant brain tumor, but its application could be hampered by low specificity due to the high false-positive rate. In this article, the authors found that no tumor cells were found in fluorescent samples from six of the 36 malignant gliomas and all four metastatic brain tumors, but false positive results were rare in the initial malignant gliomas. Using histological methods, they confirmed the sensitivity of 5-ALA for the assessment of malignant gliomas and high false-positive results in recurrent gliomas and metastatic brain tumors. It is very important to help us to do more thorough tumor removal and possibly greater safety for adjacent brain tissue. Yet, the recurrent cases, peritumoral edema, inflammatory cell and reactive astrocyte infiltration influenced the positive findings with 5-ALA. So, we need to do more studies about...
5-ALA as an aid for resection of malignant gliomas, such as how to identify the edematous tissue and necrotic tumor tissue from normal tumor tissue.

Shengde Bao, M.D.
Department of Neurosurgery
Peking University First Hospital
Beijing, P.R.C.

This excellent paper sheds light on some of the problems with the use of fluorescence guidance in the resection of malignant brain tumors. Although the concept is appealing, it is based on an incorrect premise — namely that all tumor cells will take up the fluorescent marker (in this case 5-ALA) and that normal cells will not. It is true that many tumors preferentially absorb some fluorescent markers such as HpD and 5-ALA, however, this paper demonstrates that other non-neoplastic cells may also react with this agent, particularly inflammatory cells and reactive astrocytes. Other markers have been shown to accumulate in vascular wall cells. The opposite phenomenon may also occur, where some tumor cells that are dormant or protected by the BBB may not develop fluorescence. Although the technique may have some utility, it clearly will not reliably allow for complete removal of malignant, infiltrative tumors.

Edward R. Laws, Jr., M.D., F.A.C.S.
Stanford University Medical Center
Advanced Medicine Center
Stanford, California, U.S.A.

This is an interesting report which studied histologically the resected specimens that intraoperative 5-ALA fluorescence showed false positive in 42 malignant gliomas and 6 brain metastases. The author stressed that the false positive of 5-ALA was observed more in recurrent than in initial cases, and the inflammatory reaction associated with reactive astrocyte or macrophage caused false positive findings of 5-ALA in edematous tissue. The author concluded that because intraoperative 5-ALA fluorescence is effective in initial cases of malignant glioma, the tissue surrounding tumor with vague 5-ALA fluorescence should be resected if non-eloquent, and that the surrounding tissue of the tumor was almost edematous in brain metastasis.

It can be expected that 5-ALA is taken up in the tissue with strong inflammatory reaction. However, even if the tissue with inflammatory reaction can be assumed to become false positive 5-ALA fluorescence based on this report, all tissue with vague positive 5-ALA fluorescence cannot conversely determine non-tumor tissue. The reason is that 5-ALA fluorescence may depend on the number of the tumor cells occupied in the illuminated area. There is still an unsolved issue whether the vague positive 5-ALA fluorescence can be evaluated as non-tumor tissue or not.

In brain metastasis perivascular infiltration of tumor cells is also observed in the peritumoral edematous brain tissue, so it is an important point whether the peritumoral tissue should be removed based on the findings of 5-ALA fluorescence or not. Because the inflammatory reaction is thought to appear in the necrotic part within the tumor bulk in malignant brain tumor, even if 5-ALA fluorescence shows such regions as false positive, the tumor region should be removed surgically. When intraoperative 5-ALA fluorescence shows vague findings in the illuminated tissue like this manuscript, there is always a serious problem whether those tissues should be surgically removed.

The disagreement between fluorescence or imaging diagnosis and pathological diagnosis also occurs with SPECT and MRS or PET. Quantification has also been tried to solve such disagreement, but there is still some problem with infiltrating malignant brain tumor. The solution will require further correlation with fluorescence and pathology in many specimens, as well as other good ideas in the future.

Yoshihiko Yoshi, M.D.
Department of Neurosurgery
University of the Ryukyus
Okinawa, Japan