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Confocal Microscopy for the Histological Fluorescence Pattern of a Recurrent Atypical Meningioma: Case Report

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Abstract

Background and Importance—Fluorescence-guided resection with 5-aminolevulinic acid (5-ALA), which has shown promising results in the resection of malignant gliomas, has been used for meningioma resection in an attempt to more clearly delineate the tumor margin. However, no article has investigated the fluorescence pattern of meningiomas on a histological level.

Understanding the microscopic pattern of fluorescence could help assess the precision and utility of using 5-ALA for these tumors. We present the case of a recurrent atypical meningioma operated on with 5-ALA fluorescence-guided resection for delineation of tumor tissue from surrounding uninvolved dura.

Clinical Presentation—A 53-year-old woman presented with recurrent atypical meningioma of the falx. Prior treatment included surgical resection 6 years earlier with subsequent fractionated radiation therapy and radiosurgery for tumor progression. The patient was given 5-ALA 20 mg/kg body weight dissolved in 100 mL water 3 hours before induction of anesthesia. Intraoperative fluorescence was coregistered with preoperative imaging. Neuropathological analysis of the resected falx with confocal microscopy enabled correlation of fluorescence with the extent of tumor on a histological level.

Conclusion—Fluorescence guidance allowed clear intraoperative delineation of tumor tissue from adjacent, uninvolved dura. On a microscopic level, there was a very close correlation of fluorescence with tumor, but some tumor cells did not fluoresce.

Keywords

5-ALA; Brain tumor; Contrast enhancement; Fluorescence-guided resection; Meningioma; PpIX

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Gross total resection is the goal of most brain tumor surgery when it is possible to do safely. Because intraoperative assessment of gross total resection for some tumors can be challenging, fluorescence is being used at some centers in an attempt to achieve this goal more frequently.¹⁻⁸ One fluorescence-based technology uses 5-aminolevulinic acid (5-ALA) to cause accumulation of protoporphyrin IX (PpIX) in tumor cells, which leads to emission of a red fluorescence under blue light excitation that is clearly distinct from the surrounding tissue.

Intraoperative fluorescence guidance has been used primarily for glial tumors, and early results show improved extent of resection and improved progression-free survival.^{4,9} Only a few clinical reports have described the use of 5-ALA– induced fluorescence to guide resection of meningiomas.^{10,11} In these reports, meningiomas usually fluoresce on a gross level, but the fluorescence pattern has not been investigated on a histological level. If there is a very close correlation of tumor cells with grossly visible fluorescence, then 5-ALA may give the surgeon greater confidence in the adequacy of the resection margins.

We present the case of a 53-year-old woman with a recurrent atypical meningioma, World Health Organization (WHO) grade II. This patient underwent fluorescence-guided resection using 5-ALA under an Institutional Review Board–approved Food and Drug Administration Investigational New Drug protocol.

Patient History

A 53-year-old woman initially presented 6 years earlier with a single generalized seizure. Workup included magnetic resonance imaging (MRI), and a large left anterior frontal meningioma was discovered. A left frontal craniotomy was performed, and gross total radiographic resection was obtained. Pathology revealed an atypical meningioma, WHO grade II. Less than a year later, a new area of enhancement was seen along the falx. The patient was then given fractionated radiotherapy, 50 Gy to the clinical target volume/64.4 Gy to the gross tumor volume. Four years after her radiation, the patient had recurrence along the falx. The discrete nodule was treated with 15 Gy corresponding to the 92% isodose line by linear accelerator radiosurgery. On routine follow-up MRI, increasing areas of enhancement were seen along the falx extending into both frontal lobes. She remained clinically stable, with occasional dizziness and mild headache, and was neurologically intact on examination. She was not on any antiepileptic medications and had not had a seizure since her initial presentation. Differential diagnosis at this time included a recurrent tumor with more aggressive behavior causing brain invasion or a recurrent tumor along the falx with concurrent radiation necrosis in the surrounding parenchyma.

The decision was made to operate, and the patient was enrolled in our 5-ALA protocol for brain tumor resection. The patient was given 5-ALA 20 mg/kg body weight (DUSA Pharmaceuticals, Tarrytown, New York) dissolved in 100 mL water approximately 3 hours before the induction of anesthesia. Gadolinium-enhanced, T1-weighted axial images with scalp-based registration fiducials (256 × 256 matrix, 124 axial slices, 1.5-mm slice thickness, echo time of 3 milliseconds, repetition time of 25 milliseconds; 0.1 mmol/kg body weight gadolinium-diethylenetriamine pentaacetic acid) were acquired preoperatively and

used for image-guidance navigation. A Zeiss OPMI Pentero (Carl Zeiss Surgical GmbH, Oberkochen, Germany) surgical microscope enabled for PpIX fluorescence (ie, blue light excitation and for observing PpIX fluorescence and a 620- to 710-nm band-pass filter) and a Medtronic StealthStation Treon (Medtronic, Louisville, Colorado) image-guidance navigation system were coregistered. The microscope focal point was mapped to the patient image-space coordinate system and displayed on the axial, coronal, and sagittal cross-sectional images in the StealthStation image-guidance monitor. During surgery, the surgeon switched from white light to blue light illumination modes to visualize the PpIX-induced red fluorescence (Figure 1).

Intraoperatively, 2 large nodules along the falx could be seen under white light, and both fluoresced under blue light. The surrounding dura did not show intraoperative fluorescence. Under white light, there was a clear plane between the meningioma and adjacent brain. The tumor did not appear to invade the parenchyma, which had an appearance more consistent with radiation necrosis than with invasive meningioma. Under blue light, none of the surrounding brain parenchyma displayed observable fluorescence.

Two large specimens of falx were resected en bloc, each containing 1 of the 2 fluorescing nodules and a margin of nonfluorescing, normal-appearing dural tissue (Figure 2). These specimens, coregistered with both preoperative MRI and intraoperative visualization by fluorescence under the microscope, then underwent neuropathological analysis and microscopic analysis of fluorescence patterns (Figures 3 and 4).

Tissue specimens were immediately separated into 3 equal parts for further processing as follows: 1 part was placed in formalin, a second part was placed in optimal-cutting-temperature compound and frozen in liquid nitrogen, and the third part was placed in a cryogenic vial and also frozen in liquid nitrogen. The fresh-frozen tissue part placed in optimal-cutting-temperature compound was further cut into 10- μ m tissue sections under low-light conditions.

An LSM 510 Meta laser-scanning confocal microscope (Carl Zeiss) was used for fluorescence microscopy of frozen tissue sections with a 405-nm excitation laser and a 560-nm long-pass emission filter. Low-power (0.1% laser power) laser scanning allowed acquisition of higher-quality fluorescence images by minimizing photobleaching of PpIX. Subsequently, these same frozen tissue sections were processed for hematoxylin and eosin staining. Hematoxylin and eosin images of each tissue section were acquired and registered to their corresponding fluorescence images to allow colocalization of tissue histopathology and PpIX fluorescence.

The large fluorescing nodules were in fact meningioma with radiation changes. Interestingly, the histopathological grading of this recurrence demonstrated WHO grade I features and a very low proliferative index of 1% to 2% by Ki-67 immunostaining. The surrounding, nonfluorescing dura was negative for meningioma but did show radiation changes. Microscopically, the abnormal surrounding brain parenchyma that had shown MRI enhancement did not fluoresce. Histologically, the brain parenchyma had undergone radiation changes, and no tumor was seen.

On microscopic analysis, PpIX fluorescence correlated closely with tumor tissue. Uninvolved dura showed only minimal background fluorescence (Figure 4). This correlation was very close but not a direct, 1-to-1 relationship. A thin tail of tumor on the upper left of the histological section, < 1 mm in length, did not fluoresce greater than background when examined under blue light. No areas of uninvolved dura fluoresced beyond background level.

Discussion

There have been few reports of 5-ALA use for meningiomas. Morofuji et al¹¹ reported a case of atypical meningioma with cranial invasion. In this case, the involved dura and cranial bone all fluoresced, whereas surrounding dura and bone, with no tumor microscopically, did not fluoresce. Kajimoto et al¹⁰ reported a case of a large atypical left convexity meningioma. In this case, small areas of fluorescence could be seen in the resection bed after the surgeons believed they had achieved gross total resection under white light. Furthermore, some areas of hypertrophic dura fluoresced, and tumor cells were seen on histology in these specimens. Other areas of hypertrophic dura did not fluoresce, and samples of these regions were negative for tumor. In this case report, Kajimoto et al then mention a series of 24 meningioma patients who received 5-ALA. Twenty of 24 tumors brightly fluoresced (83% sensitivity), with only tumor fluorescing in these 20 cases (100% specificity). More recently, Coluccia et al¹² published a series of 33 meningioma patients who underwent resection using 5-ALA. Thirty-one of the tumors (94%) fluoresced. One patient in this series also showed fluorescence of infiltrated skull.

A sometimes difficult aspect of the surgical management of meningiomas is determining the optimal extent of resection. Although grade I tumors are slow growing, the risk of recurrence is high if involved dura is left behind. The likelihood of recurrence increases with higher-grade tumors and increased local dural infiltration. A common practice involves taking a margin of dura around the tumor, but according to Nakasu et al,¹³ a 1-cm margin is inadequate to prevent recurrence. Ultimately, there is no Class I evidence to firmly establish the optimal dural margin needed. The ability of 5-ALA to demarcate individual tumors could be very helpful in determining the appropriate extent of dural resection, which almost certainly varies between individual tumors. However, the sensitivity of intraoperative fluorescence to pick up small foci of infiltration is not well established.

Although meningiomas do not usually have the same diffusely infiltrative nature as the glial tumors for which it has initially been used, 5-ALA may still play a valuable role in their resection. Fluorescence guidance may ensure sufficient dural margin resection and reduce the risk of tumor recurrence. Furthermore, the nonfluorescent properties of adjacent nontumor tissues (eg, vascular, neural, dural, bone) may facilitate their preservation during surgery.

In our report, the areas that fluoresced correlated very closely with tumor. Likewise, uninvolved dura did not fluoresce. Interestingly, on a histological level, this correlates with the gross results obtained by Kajimoto et al¹⁰ in which the specificity of 5-ALA fluorescence was 100% but the sensitivity was somewhat less.

Some tumor cells in this specimen did not fluoresce above background when observed microscopically. It is possible that biological variations between tumor cells that are not yet understood explain why fluorescence is seen in some cells but not others. Here, the small tail of tumor that did not fluoresce was < 1 mm in length. In practice, this provides a very useful margin for resection and may not actually be a significant limitation of the utility of fluorescence-guided resection.

The results from this patient suggest that fluorescence-guided resection with 5-ALA on meningiomas could be a useful technology for achieving the optimal extent of resection. However, analysis of more specimens is necessary to determine whether the tumor cells that do not fluoresce are seen infiltrating the dura farther away from the fluorescent tumor mass than they were in this patient. This will be the key to understanding the usefulness of 5-ALA for resection of these tumors. Long-term follow-up is also required so that results can be compared with those known for meningiomas resected by traditional techniques.

Conclusion

Fluorescence-guided resection with 5-ALA was undertaken in a case of recurrent atypical meningioma. Fluorescence guidance allowed clear intraoperative delineation of tumor tissue from adjacent, normal dura and brain parenchyma, with fluorescent areas correlating closely with tumor on histology. Because some tumor cells did not fluoresce, a number of important questions remain for future study. Further studies delineating cell-level fluorescence patterns will be helpful in understanding the ultimate utility of this technology for achieving the most accurate and safest resection of meningiomas with optimal prognostic outcomes.

Comments

This succinct case study by Whitson and coworkers explores the potential use of 5-aminolevulinic acid (5-ALA), a prodrug previously established for fluorescence-guided surgery of malignant gliomas, for fluorescence-guided resections of tumor remnants in an atypical, recurrent meningioma. Their report is not the first describing the use of 5-ALA for meningiomas; however, it is the first to incorporate confocal microscopy for correlation of fluorescence with tumor extension on a microscopic level with confocal microscopy. Their findings suggest a high level of correlation between fluorescence and tumor cell extension even though not all tumor cells fluoresced. Thus, there certainly appears to be merit in further exploring fluorescence methodology in the context of recurrent, atypical, or anaplastic meningiomas. Apart from radiotherapy, there is little in terms of therapy apart from surgery that can be offered to these patients, and more complete initial resections through the use of intraoperative fluorescence technology may make a difference.

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This case report documents the findings of an experienced group of investigators with 5-aminolevulinic acid for fluorescence-guided resection of a recurrent atypical meningioma. Fluorescence-guided resection has demonstrated efficacy for the resection of glioma in

European studies but is not yet approved for this use in the United States. There are limited data on efficacy in meningiomas, and the previous studies do not assess the correlation between fluorescence and tumor histologically.

The authors report that protoporphyrin IX fluorescence correlated closely with tumor, whereas some regions of dura and brain suspicious for recurrence based on gadolinium enhancement on magnetic resonance imaging demonstrated only radiation changes without histological evidence of tumor. Conversely, a small “tail” of tumor was observed histologically that did not fluoresce. The authors propose that a more precise understanding of tumor margin may allow a more tailored resection, which may decrease the extent of surgery-limiting complications and morbidity.

Although similar findings have been reported, this is an important study by a careful group of investigators, and both methodology and conclusions merit consideration.

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Abbreviations

5-ALA	5-aminolevulinic acid
PpIX	protoporphyrin IX
WHO	World Health Organization

FIGURE 1.

Coregistered fluorescence-guided resection of the lesion. The surgical microscope was coregistered with the navigation system, providing localization of the focal point of the microscope (ie, white cross-hairs on microscope image) with respect to the patient's magnetic resonance image (MRI) (ie, yellow cross hairs on MRIs). A contrast-enhancing nodule on MRI (A-C) is seen under white light conditions (D). Under blue light conditions (E), the nodule showed a bright pink fluorescence.

FIGURE 2.

Intraoperative images of nodule and surrounding dura. View of the specimen under white light (**A**) and blue light (**B**) with a 6-mm, bright pink fluorescent nodule and no fluorescence in the surrounding dura. Arrows with coordinate system relative to head of patient. Ant, anterior; Post, posterior.

FIGURE 3.

Images of gross permanent formalin-fixed paraffin-embedded pathology specimen (**A**) and permanent histology section (**B**), showing the lesion to be a meningioma (solid arrow) with a small infiltrating tail surrounded by normal dura (dashed arrow). Original magnification $\times 20$ (**A**) and $\times 400$ (**B**). The orientation cross allows correlation of the specimen with both the preoperative imaging and the intraoperative specimen. Ant, anterior; Post, posterior.

FIGURE 4.

Photomicrographs showing correlation of protoporphyrin IX (PpIX) fluorescence and tumor histopathology at nodule-dural tail interface. PpIX fluorescence can be appreciated only in frozen sections because further processing alters the fluorescing properties. Under $\times 100$ magnification (**A**), a clear interface between tumor (*) and normal dura (+) can be seen. A small dural tail extends out from the main tumor mass. Positive PpIX fluorescence (red) in **B** localizes to meningioma tumor infiltrates with only minimal background seen in dural connective tissues (* and + represent the same locations as in **A**). There is a short tail of tumor at the margin, < 1 mm, which does not appear to fluoresce. The specimen approximates the plane seen in Figure 1A and can be correlated using the legend.