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ORIGINAL ARTICLE

5-Aminolevulinic acid fluorescence guided resection of malignant glioma: Hong Kong experience

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Summary *Background:* 5-Aminolevulinic Acid (5-ALA) induced fluorescence is useful in guiding glioma resection. The extent of 5-ALA accumulation is beyond gadolinium contrast enhancement.[1,2] Supratotal resection may be achieved, potentially granting patients with better survival.

We present our experience on 5-ALA guided glioma resection in Chinese ethnics.
Method: Sixteen Patients ingested 5-ALA (Gliolan, Medas Germany) 20 mg/kg·m^2 4 h before surgery. The tumor resection was guided by fluorescence with neurosurgical microscope. Patient was monitored for general condition, especially for new neurological deficits. Postoperative MRI served as the assessment for extent of resection (EOR).
Result: High grade glioma was confirmed in 12 cases, low grade glioma in three and one inflammation. 5-ALA was used in ten patients with known malignant glioma, and in six patients with presumed diagnosis of malignant glioma. Fifteen cases had positive fluorescence. The intensity was strong in eight and moderate in seven cases. MRI suggested total resection was achieved in 9 patients, near total resection in two and five had subtotal resection. EOR was associated with duration between ingestion of 5-ALA and timing when microscope was brought in for visualization of fluorescence ($p = 0.038$). Two patients suffered from temporary visual field defects. One patient developed hemiparesis after surgery.
Conclusion: 5-ALA is a useful intra-operative guidance for resection. It increases the percentage of total removal of the tumor. It should be used within the window period of the action (4–12 h).

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1. Introduction

Glioblastoma remains the most malignant and most common brain tumor. A modest survival benefit was proven in the adjuvant treatment by radiotherapy[3] and temozolomide.[4] The philosophy of resecting malignant gliomas has also changed dramatically. Surgery was considered primarily diagnostic in the past. A biopsy or a conservative debulking surgery was the former practice, knowing the ultimate dismal outcome of glioblastoma. However, in recent decades ample evidences have emerged that surgical resection plays an important role in the disease outcome. In the study presented by Sinai et al, a stepwise improvement in overall survival was described once EOR was over 78%. The benefit was even more prominent within 90%—100% of the EOR range.[5] Since then, surgical resection became one of the mainstays of treating malignant glioma.

Macroscopically, it is difficult to differentiate malignant tissue from adjacent normal brain tissue. Besides, the brain shift due to craniotomy is attributive to difficulty in locating the tumor during surgery. Conventionally, the tumor bulk to be excised is considered as contrast enhancement on MRI T1 sequence. Yet glioma is notorious for its infiltrative pattern of growth, the infiltrative tumor tissue is not always in association with disrupted blood barrier. Current radiological techniques are insufficient to determine tumor boundaries. In most of the cases, the tumor tissue may extend far beyond the property of contrast enhancement.[1,2]

5-aminolevulinic acid (5-ALA) is a natural precursor of heme which can also be found endogenously. The exogenous source of 5-ALA leads to superfluous accumulation of fluorescent protoporphyrin IX in malignant glioma tissue. Its take-up in WHO grade III and IV glioma is almost 6 times more than normal brain tissue.[6] The metabolite, protoporphyrin IX can be elicited by blue light with a specific wavelength (400—410 nm) and further emits red-violet fluorescence. With a properly adjusted microscope, the luminescence can be seen clearly by neurosurgeons, serving as an excellent real-time tumor marker guiding tumor resection intraoperatively.

In the presented study, we report our experience in using 5-ALA as a surgical adjunct in resecting malignant glioma.

2. Method

2.1. Design and patients

The study was conducted in a retrospective manner. The data of those patients who had 5-Aminolevulinic Acid (Gliolan, Medas Germany) assisted glioma resection were collected and reviewed.

Patient selection criteria of using 5-ALA were as follow: known history of malignant glioma (recurrence or second resection of newly diagnosed malignant glioma); specific MRI features suggesting malignant glioma (heterogenous gadolinium contrast enhancement with or without central necrosis). Only in cases where total resection of tumor were feasible and aimed for would 5-ALA be prescribed.

5-ALA was contraindicated in patient with any one of these conditions: 1. existing fixed neurological deficit; 2. tumor infiltrating functional areas of the brain unless guided by intraoperative brain mapping; 3. only tumor debulking is opted; 4. tumor extended to ventricular system; 5. multiple, bihemispheric lesions; 6. allergy to 5-aminolevulinic acid; 7. history of porphyria; 8. pregnant patients.

All patients were sent for a pre-operative MRI scan to confirm the location of the tumor before surgery. The MRI images would be registered in neuronavigation system to enhance the accuracy of exposure during surgery. For patients with presumed diagnosis of malignant glioma, using 5ALA as an adjunct for resection was a joint decision among patients, neuroradiologists and neurosurgeons.

2.2. Surgical procedure and grading of the fluorescence intensity

5-ALA (Gliolan, Medas Germany) was prescribed to patient at a dosage of 20 mg/kg·m^2 three to 4 h before anesthesia. Craniotomy was done under traditional white light. Ultrasound and neuronavigation system were used to localize the tumor before opening dura. Surgical microscope was then brought in for excision of the tumor. Exciting blue light with an optical filter for the red light was switched on whenever the surgeon needed, especially when they encountered vague boundaries between glioma tissue and normal brain tissue. Due to the blue light's inability in penetrating tissues and also to avoid photo-bleaching, white light and blue light were used alternately during resection. Excision of the tumor was then guided by integration of all techniques including ultrasound, neuronavigation as well as 5-ALA fluorescence. After a presumed total removal of the tumor, the blue light and the filter were switched on again, ensuring that the whole cavity where the tumor had seated, was clean without any residual fluorescence. Intensity of the fluorescence was described by the in-charge surgeon as "solid", "red", "vague", "pinkish" or "no fluorescence".

2.3. Post-operative monitoring

Patients were all protected from photosensitivity in intensive care unit for at least 24 h after surgery. Neurological functions were followed-up after surgery, monthly after discharge and six months after surgery. All patients were sent for post-operative MRI within 24 h (Day 1 MRI) after surgery to determine the extent of resection (EOR). An experienced neuro-radiologist would determine the EOR by studying the pre-operative MRI and Day 1 MRI. No residual of contrast enhancement on T1 post-contrast and subtraction scans equaled to total resection. For those cases in which more than 95% of enhancement was removed, the cases would be labeled as "near total resection". More than 5% of residual enhancing signal on the Day 1 MRI was suggestive of a subtotal resection in the case.

2.4. Primary and secondary objectives

The primary outcome of the study was the percentage of patients who had brain tumors totally excised under the

guidance of 5-ALA. Secondary outcomes were the factors contributive to fluorescence intensity as well as extent of resection. Records of adverse events probably caused by prescription of 5-ALA was also retrieved, to explore the safety profile of 5-ALA. These included newly discovered neurological deficits after surgery, gastric intestinal tract upset, abnormal liver and renal functions, skin or subcutaneous tissue disorders in terms of photo sensitivity and photodermatosis.

2.5. Statistical analysis

We adopted the commonly used semi-quantitative method in grading the intensity of the fluorescence. The intensity was classified and ranked into 3 categories: a score of zero equaled to no fluorescence seen during the surgery; one referred to those cases with the surgeon's comments of "pink", "mild" or "vague"; a score of two meant the intensity was strong or the fluorescence was solid red during the procedure.

Patient's demographical data were collected. Location of the tumor, namely cortical or subcortical region, superficial or deep-seated tumor and adjacency to eloquent area were studied radiologically. With regard the pathological parameters, data on diagnosis, presence of necrotic foci (a characteristic of glioblastoma), ki-67 and MGMT promoter status were retrieved from the pathological reports. All the above-mentioned data were regarded as categorical variables.

The audit review was done in accordance with the principles outlined in the Declaration of Helsinki.

Table 1 Patient demography.

Demography	Number
Age, Mean (SD), years	48.31 (14.70)
Gender (M:F)	4:12
Index surgery	
• Newly-diagnosed glioma	6
• Recurrence	10
5-ALA intensity	
• Strong (2)	8
• Moderate (1)	7
• None (0)	1
Histology	
• Grade IV glioma	10
• Grade III glioma	2
• Low grade glioma	3
• Others*	1
Extent of resection	
•Total resection	9
• >95% resection	2
• >90% resection	5

*Inflammation.

Table 2 Operation time and some key time point in visualizing fluorescence.

Operation time	4.84
Ingestion to start using fluorescence (range)	2.20 (0.67—4.00)
End of using fluorescence (range)	6.65 (5.00—10.50)

3. Results

During 2011 to 2016, there were sixteen 5-ALA guided tumor excision cases. 5-ALA was used in ten cases with known history of high grade glioma. While in the other six cases of pre-operatively presumed malignant glioma, decision to initiate 5-ALA was based on radiological features (Table 1). Patients ingested 5-ALA 45 min to 4.00 h before start of surgery. The last inspection searching for residual fluorescence took place at 6.65 h after ingestion of 5-ALA (mean, ranging from 5.00 to 10.50 h) (Table 2). Histopathological diagnosis suggested high grade glioma in 12 cases, three low grade glioma cases and one inflammation. All cases, except one, were of positive fluorescence. The negative fluorescence case was proven to be glioblastoma. The fluorescence was strong in eight cases according to the responsible surgeons and in seven cases the intensity was moderate. Fluorescence was totally removed in eleven cases (68.75%).

Post-op MRI suggested that total resection was achieved in 9 cases (56.25%). For the rest patients, two were regarded as near total resection (>95% resection) and five were subtotal resection (>90% resection).

The intensity of fluorescence was associated with pathological markers including necrotic foci on pathological slides ($p = 0.026$, Chi-square). Extent of resection was associated with duration between ingestion of 5-ALA and timing when the surgeons started to use microscope for visualization of fluorescence ($p = 0.038$, Kruskal—Wallis) (Table 3). As for the locations of the tumor, neither superficial/deep-seated tumor nor adjacency to eloquent area was shown to have influence on EOR.

Two patients (12.5%) suffered visual field defect after surgery, both of them had spontaneous recovery at three months follow up.

One patient developed left hemiparesis during awake craniotomy monitoring due to a right deep thalamus infarct away from the resection area.

Prescription of 5-ALA was well tolerated by all patients without adverse events.

4. Discussion

The survival benefit of surgical resection of malignant glioma has long been under debate, due to the relatively inferior quality of evidence. It is difficult to carry out a randomized clinical trial to study the impacts of the extent of surgical resections on malignant glioma. The existing evidence is mostly based on retrospective studies or literature reviews. Yet these studies all suggested that extensive resection of the contrast enhancing region is beneficial for patients with malignant glioma, in terms of relieving tumor burden as well as prolonging survival. The EORTC trial also presented that the patients with totally resected malignant glioma benefited most from radiotherapy plus temozolomide.[7] All these facts are indicative that maximal

Table 3 Factors associated with extent of resection.

	Extent of resection			p-value
	Total	>95%	90%	
Superficial lesion	5	0	1	0.146*
Deep lesion	3	2	4	
Eloquent area	1	1	2	0.264*
Non eloquent area	7	1	3	
Intensity				0.728*
• Moderate	3	1	3	
• Strong	5	1	2	
Residual fluorescence				0.551*
• Yes	2	0	2	
• No	6	2	3	
Ingestion to start using fluorescence (hours)	4.59	5.00	3.00	0.038$
End of using fluorescence (hours)	7.71	7.00	5.33	0.101$

*: Chi-square; $: Kruskal—Wallis test.

safe resection of glioma holds a key role in the management of malignant glioma.

High grade glioma disrupts the integrity of the blood brain barrier, resulting in extravascular leakage of gadolinium contrast agents. This characteristic signifies MRI's importance in surgical planning especially for tumor territory. Conventionally, the tumor territory is regarded as gadolinium contrast enhancing region seen on MRI T1 post contrast sequence. While by nature, glioma grows and infiltrates into normal brain tissue, resulting in the lack of a clear demarcation between tumor and normal brain tissue. It is sometimes very difficult even for experienced neurosurgeons to tell the boundary during operation. An appreciable amount of tumor cells infiltrates around the "tumor territory", where the blood brain barrier is not yet destroyed, compromising the specificity of gadolinium contrast agent. The remnant is believed to be the culprit of future recurrence.[8]

These drawbacks can be compensated by 5-ALA. 5-ALA contrasting tumor to healthy tissue is a cellular metabolic then fluorescent process, instead of a vascular or blood brain barrier related one. Its metabolite, PpIX, is preferentially taken up then accumulates in malignant glioma cells. When shining an exciting blue light with a wavelength of 400—410 nm on to the tumor, the PpIX will subsequently emits red fluorescence. With this illumination, the surgeons can see the tumor demarcation clearly, which is meaningful for maximum resection while preserving normal brain tissue.

In the presented study, 5-ALA can be considered as useful since in 15 out of 16 surgeries the fluorescence was perceived clearly. Twelve total-fluorescent tumor removal were achieved. To avoid surgically induced neurological deficits, minimal residual of fluorescence was left intentionally during surgery in three cases. Up to 56.25% of the cohort had MRI validated gross total removal of enhancing tumor. In 68.75% of the patients the EOR was more than 95% and all patients could have more than 90% of the tumor resected. For the only case of GBM without fluorescence during operation, we believed that this was due to the too short of an interval between ingestion of 5-ALA and utilization of fluorescent guidance. The re-opening of craniotomy just took 45 min to expose the tumor, and resection was finished within 2 h.

Numerous clinical series have already proven that 5-ALA is helpful in increasing chances of total resection for malignant glioma. A German randomized controlled trial has shown that with this technique, 65% of the cases (90 out of 139 patients) had tumor totally resected, compared to 36% of the cases in which resection was done only under traditional white light. Residual volume in 5-ALA was also significantly smaller than the control group (median, 0.0 cm^3 versus 0.7 cm^3). With regards to the survival outcomes, 6-month progression free survival rate in 5-ALA group was higher than the control group (41% versus 21%). Better overall survival was granted by utilization of 5-ALA, reaching 16.7 months, nearly five months longer than its counterpart.[9,10]

One interesting finding in our study is that the interval between ingestion of 5-ALA and start of using fluorescent guidance shared a positive correlation with extent of resection. According to the product summary, when prescribing Gliolan at 20 mg/kg body weight, it takes 4 h for its metabolite protoporphyrin IX to reach a maximum plasma level. The concentration of PPIX then attenuates rapidly in the following 20 h.[11] Thus it is suggested in Western studies that 5-ALA should be taken 3—4 h before the start of surgery.[12] This interval was generally sufficient for safe induction of anesthesia and exposure of the tumor, while being just long enough to catch up with PPIX's maximum illumination. Statistically speaking, our patients ingested 5-ALA relatively later than as per instructions (mean 2.2 h versus 3—4 h before surgery). It is possible that this finding was due to an inadequate time interval for 5-ALA to exert its best performance. All the patients in our center were to be monitored in intensive care unit after surgery for at least 24 h to be protected from photosensitivity as well as to monitor their neurological functions.

We also had some concerns about whether the so-called "no residual fluorescence" after prolonged surgery was due to totally metabolized/bleached PpIX, instead of being attributive to totally resected illumination. Although our study could not clarify the query, it is important to find that in some cases when the surgeons were doing the final inspection with the optical filter, the fluorescence could still be seen clearly 8.0—10.5 h after ingestion of 5-ALA. Similar results were presented by Stummer et al in their 1998 study. They reported that although the tumor resection did not last for more than 8 h after 5-ALA administration, the tumor fluorescence was still highly discernible at the end of the procedure. The dosage used for their patients was only half that of the 20 mg per kilogram body weight.[12] According to Colditz et al, the intensity of fluorescence was not inferior even at 12—16 h post-ingestion of 5-ALA.[13] Then the importance of our finding, that 5-ALA should be used in the window period of action (4—12 h after ingestion) stands out. Surgeons sometimes concern that once the surgery is delayed, the fluorescence starts to decay, the reliability of "total excision of fluorescence" in indicating entire removal of tumor is then compromised just like our initial concern. But our study, as well as the above-mentioned studies, suggested that the fluorescence impression is unperturbed even beyond 12 h. In this case, "unexpectedly

delayed cases", could actually be granted with equally or at least non-inferiorly expectable outcome, in terms of potentially superior extent of resection. But indeed, these hypotheses need to be proved by a randomised controlled trial recruiting a much larger population.

Since the idea of using 5-ALA as an adjunct is based on a cellular mechanism, some scientists explored the correlation between intensity of fluorescence and tumor cellularity. The settings of these studies were similar. Several intraoperative biopsies were taken at tumor areas with different fluorescent intensity for histology exam. These studies all reached similar conclusions that intensity had positive correlation with tumor cellularity, ki-67/MIB1 index.[14–16]

The above-mentioned studies also discussed the predictive ability of 5-ALA induced fluorescence. The negative predictive value of absent fluorescence was not as superior as the positive predicting value of presence of fluorescence. Despite the positive and strong fluorescence accurately demonstrates tumor core, the absence of fluorescence could only predict absence of tumor in 30%–66% of the cases.[14,15,17]

There are two obstacles hindering neurosurgeons from performing maximal surgical resection of glioma for all patients: resectability and localization of the tumor. Although the tumor territory could be seen clearly on the pre-operative MRI, it is difficult even for experienced neurosurgeons to tell malignant tissues from adjacent normal brain tissues intraoperatively with naked eyes. Neuronavigation system is helpful in pre-operative planning, but its accuracy is compromised by brain shift after craniotomy and opening of the dura. Ultrasonography is real-time but the discrimination effect at the tumor brain interface is still vague and poor. Intra-operative MRI is not available in most of the hospitals. So, surgical techniques, navigation, ultrasonography, 5-ALA fluorescence guidance and mapping, are all needed for safe maximal resection.

The limitations of this study are clear. This paper is a small-scale retrospective study, based on our experience in using 5-ALA during 2011–2016. Due to the retrospective nature of the study, we did not have sufficient data to do the analyses regarding cellularity and tumor markers such as ki-67/MIB-1. 5-ALA is a potentially toxic agent to liver and kidney. All the patients need extra protection to their eyes and skin from light after the surgery, thus it is not recommended routinely to glioma patients.

Another reason for the relatively small sample size is that this surgical adjunct is not for everyone with brain tumor. For example, patient with fixed neurological deficit (unless with awake mapping), patient who can have the tumor only partially resected is contra-indicated for giving 5-ALA. Close proximity to the functional area or ventricle also limits the application of 5-ALA in patients. So in this paper, we were actually looking into a relatively seldom used technique assisting the surgery on a relatively uncommon disease for Asian population. The related report is also not often seen in Asia wide territory.

To conclude, 5-ALA is a useful intra-operative guidance for resection. It will increase the extent of resection, percentage of gross total removal and even supra-total removal of the tumor. Moreover, it should be used within the window period of the action (4–12 h).

Declaration

This study was approved by the Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethic Committee (CRE Ref Number: 2017-314). This study was done in accordance with the principles outlined in the Declaration of Helsinki.

Conflict of interest

All authors declared non-conflict of interest.

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References

1. Stummer W, Stepp H, Möller G, Ehrhardt A, Leonhard M, Reulen HJ. Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue. *Acta Neurochir (Wien)*. 1998;140(10):995–1000. http://dx.doi.org/10.1007/s007010050206.
2. Roberts DW, Valdés PA, Harris BT, et al. Coregistered fluorescence-enhanced tumor resection of malignant glioma: relationships between δ-aminolevulinic acid–induced protoporphyrin IX fluorescence, magnetic resonance imaging enhancement, and neuropathological parameters. *J Neurosurg*. 2011;114(3):595–603. http://dx.doi.org/10.3171/2010.2.JNS091322.
3. Walker MD, Green SB, Byar DP, et al. Randomized comparisons of radiotherapy and nitrosoureas for the treatment of malignant glioma after surgery. *N Engl J Med*. 1980;303(23):1323–1329. http://dx.doi.org/10.1056/NEJM198012043032303.
4. Stupp R, Mason WP, Van Den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352(10):987–996. http://dx.doi.org/10.1056/NEJMoa043330.
5. Sanai N, Polley M-Y, McDermott MW, Parsa AT, Berger MS. An extent of resection threshold for newly diagnosed glioblastomas. *J Neurosurg*. 2011;115(1):3–8. http://dx.doi.org/10.3171/2011.2.JNS10998.
6. Gliolan. *INN: 5-aminolevulinic acid hydrochloride*. United Kingdom: European Medicines Agency; 2010.
7. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol*. 2009;10(5):459–466. http://dx.doi.org/10.1016/S1470-2045(09)70025-7.
8. Chang EL, Akyurek S, Avalos T, et al. Evaluation of peritumoral edema in the delineation of radiotherapy clinical target volumes for glioblastoma. *Int J Radiat Oncol*. 2007;68(1):144–150. http://dx.doi.org/10.1016/j.ijrobp.2006.12.009.
9. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen H-J. Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial. *Lancet Oncol*. 2006;7(5):392–401. http://dx.doi.org/10.1016/S1470-2045(06)70665-9.
10. Stummer W, Reulen H-J, Meinel T, et al. Extent of resection and survival in glioblastoma multiforme. *Neurosurgery*. 2008;62(3):564–576. http://dx.doi.org/10.1227/01.neu.0000317304.31579.17.

11. *Scientific Discussion 5 ALA*. 2007.
12. Stummer W, Stocker S, Wagner S, et al. Intraoperative detection of malignant gliomas by 5-aminolevulinic acid- induced porphyrin fluorescence. *Neurosurgery*. 1998;42(3):518—526. http://dx.doi.org/10.1097/00006123-199803000-00017.
13. Colditz MJ, Van Leyen K, Jeffree RL. Aminolevulinic acid (ALA)-protoporphyrin IX fluorescence guided tumour resection. Part 2: theoretical, biochemical and practical aspects. *J Clin Neurosci*. 2012. http://dx.doi.org/10.1016/j.jocn.2012.03.013.
14. Stummer W, Tonn JC, Goetz C, et al. 5-Aminolevulinic acid-derived tumor fluorescence: the diagnostic accuracy of visible fluorescence qualities as corroborated by spectrometry and histology and postoperative imaging. *Neurosurgery*. 2014. http://dx.doi.org/10.1227/NEU.0000000000000267.
15. Díez Valle R, Tejada Solis S, Idoate Gastearena MA, García De Eulate R, Domínguez Echávarri P, Aristu Mendiroz J. Surgery guided by 5-aminolevulinic fluorescence in glioblastoma: volumetric analysis of extent of resection in single-center experience. *J Neurooncol*. 2011. http://dx.doi.org/10.1007/s11060-010-0296-4.
16. Coburger J, Engelke J, Scheuerle A, et al. Tumor detection with 5-aminolevulinic acid fluorescence and Gd-DTPA-enhanced intraoperative MRI at the border of contrast-enhancing lesions: a prospective study based on histopathological assessment. *Neurosurg Focus*. 2014;36(2). http://dx.doi.org/10.3171/2013.11.FOCUS13463. E3.
17. Lau D, Hervey-Jumper SL, Chang S, et al. A prospective Phase II clinical trial of 5-aminolevulinic acid to assess the correlation of intraoperative fluorescence intensity and degree of histologic cellularity during resection of high-grade gliomas. *J Neurosurg*. 2016;124(124):1300—1309. http://dx.doi.org/10.3171/2015.5.JNS1577.