Fluorescence-guided Resection of Malignant Gliomas Using 5-aminolevulinic Acid:

Practical Use, Risks, and Pitfalls

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The need for novel, effective, and safe treatments for malignant glioma is obvious. Despite surgery, radiotherapy, and chemotherapy, the median survival time of patients with glioblastoma multiforme (World Health Organization Grade IV) still does not exceed 15 months. Patients with anaplastic astrocytoma (World Health Organization Grade III) have a slightly better prognosis.

As a result of the infiltrative nature of these tumors, there is controversy about the usefulness of gross total resection. Nevertheless, in the view of most neurosurgeons, surgical cytoreduction of tumor and adjacent tissue containing infiltrating cells of high density is an accepted treatment modality for these tumors. For malignant gliomas, an important aim is the safe removal of all contrast-enhancing tumor. In the past, this has been viewed to be a major factor determining progression and, ultimately, survival.[1,2,6,13] Furthermore, emerging novel adjuvant therapies such as immune therapy, gene therapy, or strategies for chemotherapy will most likely rely on maximal cytoreduction to be as effective as possible. In this context, it was observed that concomitant radiochemotherapy followed by adjuvant chemotherapy was most efficacious in patients treated by complete resections as compared with incomplete resections or biopsy.[18]

However, the goal of removing all contrast-enhancing tumor on magnetic resonance imaging (MRI) has traditionally been reached only in less than 30% of cases[1,5,12] because viable tumor tissue at the margin of a resection cavity is often difficult to distinguish intraoperatively. Therefore, a number of technical adjuncts to surgery, such as intraoperative MRI,[9] neuronavigation,[20] and ultrasound,[7] have been explored in the past.[7] Moreover, MRI is expensive, neuronavigation has a problem of brain shift, and ultrasound is prone to artefacts and problems of image interpretation. Few data exist from prospective randomized trials to prove efficacy. Integration of diffusion tensor-weighted imaging and fiber tract imaging into resection planning for gliomas led to improved functional outcome with the benefit for survival.[21] However, systematic reviewing of radicality of resection was not a primary end point of that study. Willems et al. conducted a small randomized trial to investigate the usefulness of neuronavigation in glioma surgery in view of procedural aspects and functional outcome.[19] They could not detect survival benefit for the additional use of neuronavigation, possibly as a result of the small sample size (45 patients) and short follow-up (3 months). Therefore, none of these methods has evolved into a standard for surgery on malignant gliomas with the aim of optimizing resection.

Recently, 5-aminolevulinic acid (ALA) has emerged as a drug with great practical usefulness as a metabolic marker of malignant glioma cells that can be used intraoperatively for identifying residual tumor. ALA is the body's own metabolite in the heme biosynthesis pathway. Experimental and clinical studies have shown ALA to be taken up by malignant glioma cells, where it is converted into strongly fluorescing porphyrins.[16] Using specifically modified surgical microscopes, the resulting fluorescence can be used for resecting residual malignant glioma tissue (*Fig. 3.1*).[13,15] Fluorescence-guided resections using ALA have since been tested for efficacy and safety in a prospectively randomized setting.[14] The study was able to demonstrate early postoperative MRI to be devoid of residual, contrast-enhancing tumor in 65% of patients with glioblastoma multiforme in the ALA group compared with only 36% in a conventional microsurgical control group. Kaplan-Meier analyses revealed significantly prolonged progression-free survival in patients on ALA compared with white light alone with cumulative 6-month progression-free survival rates of 41 and 21% ($P < 0.01$), respectively.

Because of its benefits and an acceptable safety profile, marketing approval for ALA has recently been granted by the European Medicines Evaluation Agency for use in the European community in adult patients for visualization of malignant tissue during surgery for malignant glioma (World Health Organization Grades III and IV).

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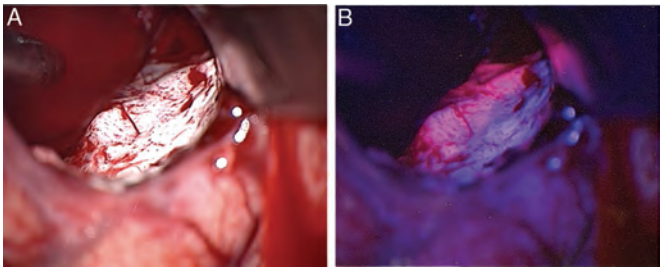

FIGURE 3.1. Intraoperative photograph of tissue marginal to glioblastoma multiforme in a patient after prior administration of 5-ALA. *A,* Inconspicuous appearance under conventional illumination. *B,* Vivid red porphyrin fluorescence marking residual malignant glioma tissue.

This article provides an overview of the practical use of ALA as well as pointing out the avoidance of possible pitfalls and risks of fluorescence-guided surgery.

Dosage and Time of Aminolevulinic Acid Application

Large-scale clinical applications of fluorescence-guided resection using ALA have only been performed and tested using an oral dose of 20 mg 5-ALA/kg body weight in combination with dexamethasone (3 × 4 mg per day for 2 d). This level of dexamethasone pretreatment is believed to be crucial for the efficacy and safety profile observed in the Phase III study, because dexamethasone very likely influences ALA uptake and fluorescence yields by tightening the blood–brain barrier. Lower doses (0.2 and 2 mg/kg) have been tested in a double-blind, prospectively randomized trial (medac MC-ALS.8-I/GLI, company-supported clinical Phase I/II study; medac GmbH, Wedel, Germany, unpublished data) but were found to generate considerably less fluorescence and were thus abandoned. That same study revealed no differences in the frequency of adverse and severe adverse events among the three doses. Higher doses have not been tested for fluorescence-guided resections of malignant gliomas of the brain. However, systemic side effects with higher doses of 5-ALA, like those used for other indications, have been reported. Doses of 40 mg/kg body weight or more have been observed to induce hypotension, nausea, and vomiting and increases in plasma liver enzymes.[3,4,8,11] On the other hand, because protoporphyrin IX fluorescence using 20 mg/kg body weight has proved adequate for efficient detection and/or resection of malignant gliomas, there does not appear to be any necessity for increasing fluorescence intensity by augmenting the 5-ALA dosage.

In practice, 5-ALA is given to patients orally 2.5 to 3.5 hours before induction of anesthesia. The time point of administration was derived from initial in vivo experiments using the C6 glioma model in rats. In these experiments, maximal porphyrin fluorescence was observed approximately 6 hours after administration. Fluorescence was less after 3 and 9 hours.[16] For surgery on patients, it was calculated that induction of anesthesia, positioning of the patient, draping, and craniotomy would take approximately 1.5 to 2 hours. Allowing another hour for removal of the clearly discernible tumor core, the surgeons would be 3 to 3.5 hours into the operation before fluorescence guidance becomes necessary. Hence, administration should be timed for 3 hours before surgery. Before application, the compound is easily dissolved in tap water and should be freshly prepared to rule out degradation. In our practice, for a dose of 20 mg/kg, 1.5 g (the contents of a vial of lyophilized compound) is dissolved in 50 mL of tap water and given to the patients. If additional 5-ALA is necessary, the contents of a second vial have to be dissolved.

It is common practice to maintain complete restriction of food and drink for at least 6 hours before general anesthesia. Pharmacokinetic assessments performed in the medac clinical Phase I/II study (MC-ALS.8-I/GLI, unpublished data) have demonstrated complete absorption of the total 5-ALA dose within 1 hour of oral administration so that the solution can be expected to have passed the intestines at the time point of anesthesia induction. Accordingly, complications arising from anesthesia induction after recent 5-ALA administration have as yet not been recorded.

In normal clinical practice, the time limit of 2.5 to 3.5 hours for administration cannot always be exactly maintained. In the case of unforeseen delays, for instance, as a result of blocking the operating room by unscheduled, intervening emergency surgery, it would not be recommended to cancel the fluorescence-guided procedure and to reschedule it for another day. It is unknown what side effects short-term re-administrations of 5-ALA would elicit. Fortunately, fluorescence in human malignant gliomas appears to be very stable. In our experience in similar situations, an unperturbed fluorescence impression was obtained even 12 to 16 hours after administration of 5-ALA. The fluorescence did not appear to be inferior to the fluorescence obtained in tumors operated on within the usual time range.

Light Protection

Sensitization of the skin has been reported after systemic application of 5-ALA.[3,8,11] In contrast to traditional sensitizers, fortunately, skin sensitization resulting from 5-ALA is short, with a duration of approximately 24 hours. Consequently, when using 5-ALA in clinical practice, this temporary side effect has to be considered. In our practice and as previously recommended for patients in the Phase III ALA trial, direct exposure of patients to sunlight or strong room light has to be avoided for 24 hours. Between the time point of 5-ALA administration and induction of anesthesia, low levels of ambient light are permitted. In our opinion, the most vulnerable period is during the induction of anesthesia and the positioning of the patient for surgery, before draping. During this phase of bustling activity in the neurosurgical operating room, direct illumination by overhead lighting is

often unavoidable. We try at least to prevent illumination of the patient's skin by the operating lights. Low levels of ambient light are permitted during the postoperative period. Restrictions regarding light exposure are withdrawn 24 hours after 5-ALA administration. Observing these precautions, no severe skin reactions in terms of significant sunburn have been observed, although several patients have had slight rubor of the skin lasting 2 to 3 days.

Intraoperative Photosensitization of Brain Tissue

During the initial application of 5-ALA for treating patients, there was some concern about possible photosensitization of normal brain elicited by the surgical microscope light source. However, spectrographic studies have since confirmed that with 20 mg/kg in the steroid-pretreated patient, no 5-ALA-dependent porphyrin fluorescence is detectable within normal or edematous, perifocal human brain[13] (medac clinical Phase I/II study MC-ALS.8-I/GLI, unpublished results). Experimentally, 100 mg of 5-ALA/kg in rats did not result in sensitization of normal brain tissue. In rats edema model, on the other hand, slight sensitization was observed with 100 mg 5-ALA per kilogram body weight with damage detectable to a depth of less than 1 mm using a 635-nm diode laser and 200 J/cm^{-2} illumination energy. Conversely, the traditional photosensitizer Photofrin II (Axcan, Mont-Saint-Hilaire, Canada) was found to nonspecifically sensitize normal cortex even in the absence of edema,[10] leading to greater damage than 5-ALA pretreatment in the face of brain edema. Illumination densities obtained with conventional surgical microscopes (like the Zeiss NC4; Carl Zeiss AG, Oberkochen, Germany, which had been used in the Phase III trial) are typically in the range of 40 to 80 mW/cm^{-2} and strongly depend on microscope distance from the resection cavity. Even in this setting, it would take more than 30 minutes to reach energy levels consistent with those used for phototherapy of malignant gliomas. Taken together, our experience and experimental results fail to demonstrate any risk of damage to normal brain during fluorescence-guided neurosurgery by phototoxic mechanisms.

Equipment

Adequate equipment for the visualization of porphyrin fluorescence is of utmost importance for successfully implementing fluorescence-guided resections. In modern neurosurgical practice, the surgical microscope is indispensable. Therefore, fluorescence imaging hardware has to be adapted to the microscope. In its simplest form, fluorescence could be visualized by introducing a short-pass filter into the excitation light path to filter out the proper excitation wavelength, which is shorter than the fluorescence emission wavelength. A long-pass filter placed into the observer light path could block out excitation light and allows only red porphyrin-induced fluorescence to pass. However, "monochrome" imaging conducted in this fashion would not allow recognition of tissue outside of fluorescing tumor and would therefore not be suited for neurosurgical applications. All light-giving tissue detail would be blocked out by the observation filters. For this reason, the microscope features a refined combination of excitation and emission filters with slightly overlapping transmission,[15] a combination developed by researchers at the Laser Research Laboratory, Klinikum Grosshadern, Munich. As a result of the overlap, a small fraction of the excitation light is remitted from tissue and gives a normal brain a blue tone in contrast to bright red porphyrin fluorescence. The degree of filter overlap is crucial for successful imaging. If remitted light is too strong, porphyrin fluorescence is no longer recognizable. If remitted light is too weak, surgery becomes difficult as a result of the lack of tissue detail. Also, faint autofluorescence in the red spectral region becomes visible and can be mistaken for porphyrin fluorescence. In the first case, sensitivity of detection would be impaired; in the second case, specificity may be reduced, a worrisome effect with possible danger to the patient as a consequence of unwanted resections.

In the medac study MC-ALS.3/GLI (comparative, unblinded Phase III trial),[14] only the Zeiss NC4 system with its particular combination of excitation light source, excitation filter, microscope optics, and emissions filters was tested for safety and efficacy. All participating centers were equipped with identical microscopes. Whether the results from this study can be extended to equipment supplied by alternate microscope manufacturers is unknown. If alternate equipment was more sensitive, it might pick up tissue autofluorescence or diffuse porphyrin fluorescence not seen with the Zeiss system leading to greater resection volumes, which might jeopardize safety. If alternate equipment was less sensitive, this might result in less radical resections. Thus, caution is warranted in this regard.

Finally, to visualize fluorescence on a video screen for documentation purposes, specially modified video cameras are necessary. One such camera, which was used in the Phase III study, was a three-chip color CCD camera from Karl Storz, Tuttlingen, Germany (Tricam SL PDD PAL). This camera was optimized for red porphyrin fluorescence detection by enhanced sensitivity in the wavelength range beyond 600 nm. The camera circuitry enabled automatic or manually adjustable target integration of the porphyrin signal with exposure periods ranging from 1/10,000th of a second to 2 seconds. Typical exposure times are 1/15th to 1/30th of a second. When switched to the fluorescence mode, the gain of the red channel was automatically increased relative to the other channels (blue and green) to increase red sensitivity.

During surgery, however, it is wise to rely on the impression directly obtained with the eye, which has the greatest range of perceiving differences in fluorescence intensity and distinguishing fluorescing from nonfluorescing tissue. With the cam-

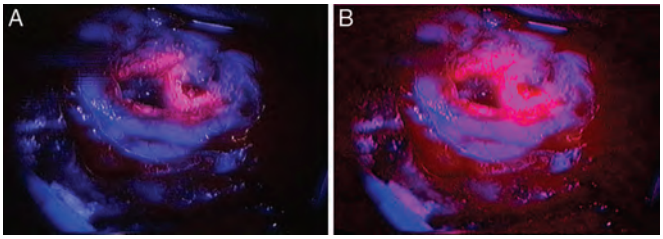

FIGURE 3.2. Over-amplification or over-integration of video signal generated during fluorescence-guided resection. Blue, inconspicuous neighboring tissue adapts red fluorescence, which represents over-amplified autofluorescence and not porphyrin fluorescence. *A,* normal video view; *B,* overamplified image.

era system, it is possible to increase gain and integration arbitrarily for fluorescence to appear more intense. However, unaffected brain tissue has a slight red autofluorescence, which is not perceived under normal conditions or with the eye. Undo amplification of the camera signal will cause normal tissue to appear vaguely fluorescent (*Fig. 3.2*).

Implementation During Surgery

Apart from necessary precautions regarding light exposure, the course of the normal operation does not differ greatly from conventional microneurosurgical operations. Anesthesia induction, patient positioning, draping, and craniotomy are performed in the usual way. Like in other operations, neuronavigation can be a useful adjunct for planning craniotomy or locating tumors, which do not reach the cortical surface. Alternately, for initial localization of tumor, sonography may be used. At times, even when the cortical surface appears normal, switching to blue excitation light may allow discrimination of subcortical tumor extensions, providing a valuable guide for initial corticotomy (*Fig. 3.3*). For surgery in eloquent brain regions, we have repeatedly used awake craniotomy for language mapping or for surgery in the vicinity of the motor cortex, and this has never interacted negatively with fluorescence-guided resections. Apart from initial localization, neuronavigation or sonography has never been of additional help for defining tumor borders. However, sonography has been helpful in defining gross anatomy, for instance when operating on temporal tumors extending toward the basal ganglia. In this situation, sonography is useful for delineating the sylvian fissure and insula to define the plane for termination of resection before entering the basal ganglia. It must be borne in mind that tissue fluorescence only provides two-dimensional information on tumor extensions and does not prevent the surgeon from following the tumor into eloquent brain. Although intact neurological function does not seem likely within fluorescing tissue, surgical manipulations may lead to remote damage away from the immediate resection site, for instance by damaging blood vessels traversing the tumor. Therefore,

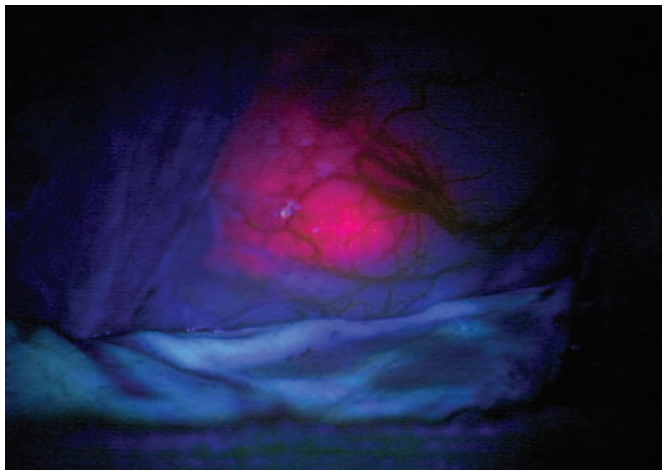

FIGURE 3.3. Tumor fluorescence shining through the cortex allowing precise planning of initial corticotomy for reaching the tumor.

5-ALA is a tool that helps discriminate tumor, but it is at the surgeon's discretion as to whether all the fluorescing tissue encountered should be removed.

There are of course different methods for operating on a malignant glioma. The surgeon might prefer to remove necrotic and easily distinguishable solid tumor regions first, predominantly under white light, and then to remove marginal, residual tumor using fluorescence-guided resection. Alternately, he or she might choose to remain in fluorescing tumor margins at the resection plane. We use simple suction or the ultrasound aspirator for removing tumor. The monopolar loop, which is not a common microsurgical tool, has to be avoided in ALA-assisted surgery, because its use destroys tissue fluorescence. By sucking away nonfluorescent tissue debris, vital fluorescing tissue is re-exposed, which may then be resected. Blood in the resection cavity quenches the fluorescence signal but can easily be removed by suction to give an impression of the fluorescence quality of the tissue. In this respect, fluorescence-guided resection is a dynamic process. Conditions do not have to be optimized for demonstrating fluorescence. Rather, switching from normal to blue excitation light in the microscope is performed manually and repeatedly during the course of the operation. Toward the end, longer periods of the operation can be performed using blue excitation light alone. If unspecific oozing gets too strong and impairs fluorescence detection, white light illumination with its greater detail is used for coagulating vessels.

Photobleaching

There has been some concern about photobleaching of porphyrins by microscope illumination, which might destroy tissue fluorescence and impair sensitivity. We have examined this issue in detail in earlier work[17] and found photobleaching to be much slower than anticipated. Under operating light

conditions, fluorescence decayed to 36% in 25 minutes for violet–blue and 87 minutes for white light. Moreover, during surgery, microscope light is usually directed at a small part of the resection cavity, whereas other parts are often covered by coagulated blood or cotton patties. Still, mild fluorescence may be bleached in exposed regions of the tumor, which are not removed immediately and may be missed. In this situation, fluorescence may be refreshed by suction and removal of superficial cell layers.

How Far to Resect

Past investigations (medac clinical Phase II study MC-ALS.28/GLI, unpublished data) have demonstrated the extent of 5-ALA-derived fluorescence accumulation to exceed the area of contrast enhancement observed on MRI. Thus, ALA-derived fluorescence appears to be more sensitive in delineating residual tumor. MC-ALS.28/GLI has further demonstrated sensitivity to be limited to a density of infiltrating tumor cells of approximately 20%. It is reasonable to assume that infiltrated brain tissue in these regions might be functionally deficient; however, this cannot be assumed in general. Furthermore, slight disruption of the blood–brain to small molecules such as 5-ALA will have to be expected in fluorescing tissue regions. Disruption of the blood–brain regarding small molecules is a prerequisite for ALA uptake into the brain. Disruption will also preclude a normal milieu intérieur with intact function. On the other hand, going beyond this region might lead to neurological deficits. It is also evident that tissue manipulation such as coagulation or shearing might result in damage to neighboring brain regions with consequent neurological deficits. Furthermore, blood vessels supplying adjacent, eloquent blood regions might be damaged, leading to distant ischemia, again resulting in neurological deficits. It is important to bear in mind that ALA-derived fluorescence is a tool for delineating tumor. It is the responsibility of the surgeon to decide how far he or she is prepared to remove fluorescing tissue. ALA should not replace critical awareness of cerebral anatomy, vascular supply, and function of the brain region, in which resections are performed.

In the medac Phase III ALA trial, there was a slight preponderance of neurological deterioration in the ALA arm 48 hours after the procedure.[14] Further analysis demonstrated deterioration to have occurred almost exclusively in patients having preoperative neurological deficits that were not completely responsive to steroid pretreatment. Steroid pretreatment was obligatory within that trial. This observation strongly cautions against attempts at "radical" resection in such patients, whose tumors have already caused structural damage in eloquent brain regions, rather than impaired function through edema alone.

In the authors' experience, high-energy ultrasonic aspirator resections can be performed for strongly fluorescing tumor, whereas low-power ultrasonic aspirator settings are desirable when resecting marginal tumor with weak fluorescence. In eloquent regions (motor cortex, speech), the authors prefer awake craniotomies and cortical or subcortical stimulation to minimize the risk of neurological deficits when operating in critical regions.

Pitfalls

In some of the studies on fluorescence-guided resections using ALA, incompletely resected tumors were observed in which anatomical location did not preclude complete resection. An analysis of the images pertaining to these patients revealed a common problem, the problem of overhanging margins (*Fig. 3.4*). During the course of resecting tumors, surgeons tend to undercut the cortex, leaving residual tumor under the margins, that is, outside the surgeon's direct field of vision. It is important to be aware of this phenomenon to prevent missing substantial portions of the tumor. A second problem can frequently be encountered in tumors with cystic portions and slender margins of enhancement. Opening the cysts leads to the collapse of parts of the tumor, which in some cases might be missed. Finally, wrongly placed craniotomies have been identified as the main factor precluding complete resection of contrast-enhancing tumor.

Other pitfalls were observed when patients' histology did not conform to the expected malignant glioma, for example, when they sustained abscess, metastasis, vasculitis, and lymphoma. In the experience of the authors, both abscesses and metastases are sometimes surrounded by a region with weak, unspecific fluorescence accumulation. Although the source of this fluorescence is unclear, it is crucial to be aware of this phenomenon to avoid fluorescence-guided resections

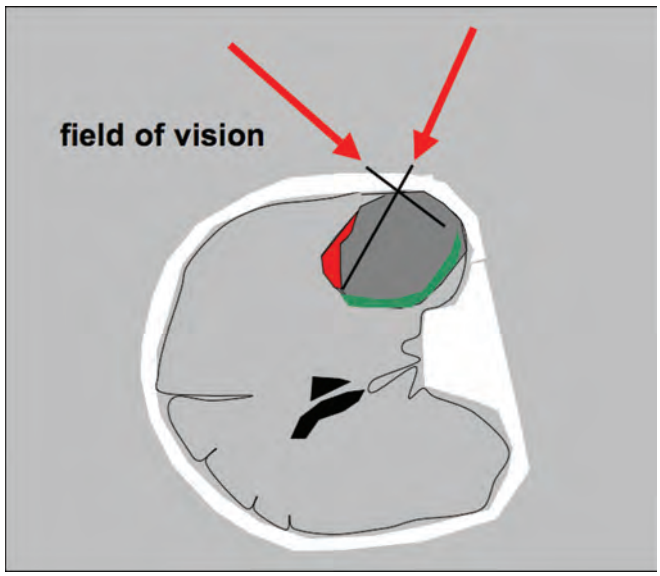

FIGURE 3.4. Overhanging edges may obscure the line of vision to residual tumor and provisions should be made to visualize these regions also.

in perifocal tissue in these entities. Patients with lymphoma and necrotizing vasculitis were also observed to demonstrate strong fluorescence accumulation within their lesions, possibly related to inflammatory or neoplastic cells. Because of this experience, we recommend obtaining frozen sections at an early stage of the procedure in any unclear cases to prevent unnecessary damage in nonsurgical lesions. Similarly, stereotactic biopsy might be performed ahead of definite surgery to clarify diagnosis in ambiguous MRIs. Finally, some cases of gliosarcoma have been observed to show only modest fluorescence accumulation within those areas of the tumor with predominantly solid texture, although infiltrating tumor beyond that had revealed the expected fluorescence.

Apart from incorrect histology, a number of technical reasons for missing fluorescence have been identified such as the drug simply not having been administered or having been administered with too much delay without the surgeons having been informed. With the early prototype microscopes used in the medac Phase III ALA study[14] (Carl Zeiss NC4 OPMI Fl), the sled responsible for sliding the fluorescence filter into the observer light path was sometimes defective. In these cases, the lamp changed wavelengths correctly yet no fluorescence could be visualized because excitation light was not blocked out. Therefore, before draping the microscope, it is imperative that the system be carefully tested to identify malfunction early and to prevent undue delays during the procedure. Fluorescence that is too weak has been related to overaged xenon lamps, because as the lamps age, they lose intensity. Furthermore, illumination geometry plays a role. There is an exponential decline of excitation light intensity with growing distance; thus, distance should be kept small, if feasible (*Fig. 3.5*). Furthermore, if resection is performed through a small corticotomy, the light reaching into the cavity may be limited (*Fig. 3.5*). It is common experience that with ongoing resection, the fluorescence signal appears to increase, but this is probably related to progressive widening of the approach corridor within the cortex. Positioning the microscope so that the illumination light is as perpendicular to the resection surface as possible will also increase fluorescence yields.

Finally, ambient light within the operating room will interfere with the fluorescence signal. Neon lighting contains substantial red and infrared light. This will be filtered selectively by the filters in the microscope in the observation light path and will make the entire cavity and brain appear red. Designated operating room lights are usually filtered in the red and infrared wavelengths to reduce heat transmission and do not generally pose a problem. During our procedures, we usually switch off all neon illumination, whereas surgical lights are directed away from the cavity.

During surgery, care must be taken to reduce ambient room light. Normal room lighting, especially neon tubes, has a strong red component. Red wavelengths are selectively amplified by the detection equipment and thus lead to red discoloration of nontumor tissue normally perceived as being blue. Standard surgical lights are less of a problem in this regard, because their red and infrared wavelengths are blocked more effectively. In our practice, we turn off all light sources except the surgical lights, which are diverted away from the surgical cavity toward the instrument trays.

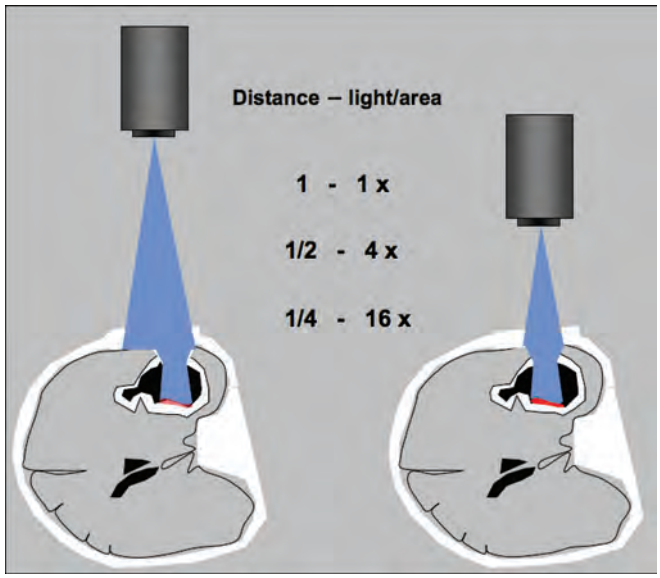

FIGURE 3.5. Scheme demonstrates the exponential decrease in light with growing distance to fluorescent tissue, resulting in weak fluorescence intensity. Furthermore, small corticotomies shade illumination light and also result in low fluorescence intensities, especially if distances are large.

We have found it helpful to reduce light intensity under white light to aid fast adaptation to violet–blue excitation light. Modern neurosurgical microscopes such as the Zeiss, Germany NC4 or Pentero systems have excessive white light in any case and reducing this by 50 to 70% does little to reduce image quality under white light. This is especially important for the Pentero system, which features an excess of normal white light, the intensity of which can easily be reduced to 25%. Even in standard neurosurgical procedures, it is wise to reduce illumination intensity (which in addition reduces thermal stress on the tissue as well) and to reserve the full capabilities of this system for deep-seated procedures and/or procedures with high magnification. For fluorescence-guided resections, however, the system should automatically switch to full intensity to have the highest possible intensity of violet–blue excitation light.

CONCLUSION

Fluorescence-guided surgery using ALA is a simple tool for identifying residual malignant glioma tissue intraoperatively. However, to achieve optimal results, a number of pitfalls and risks and their avoidance have to be kept in mind.

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