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Technical Principles for Protoporphyrin-IX-Fluorescence Guided Microsurgical Resection of Malignant Glioma Tissue

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Summary

Malignant gliomas accumulate fluorescing protoporphyrin IX intracellularly after exposure to 5-aminolevulinic acid, a metabolic precursor of haem. This phenomenon has been exploited for intra-operative identification of residual tumour to enable greater completeness of tumour removal. The present report describes the necessary modifications to the operating microscope to enable microsurgical, fluorescence-guided tumour removal.

The system consists of a xenon light source coupled to the microscope, which can be switched from normal white light to violet-blue excitation light (375–440 nm). A longpass filter is introduced into the observer light path to enable observation of tumour fluorescence. Transmission characteristics of excitation and observation filters are chosen to transmit part of the remitted excitation light. Thereby the observer retains an impression of tissue detail, next to tumour porphyrin fluorescence. An integrating three chip CCD camera optimized for red light detection enables documentation of fluorescence findings.

The present modifications allow uncomplicated and rapid recognition of red tumour fluorescence and its borders to normal tissue, without interrupting the course of the operation. Tissue detail is great enough to enable tumour resection under violet-blue excitation light during parts of the operation. The system appears to constitute a useful tool for optimizing removal of malignant gliomas on a routine basis.

Keywords: Aminolevulinic acid; malignant glioma; fluorescence detection; fluorescence microscopy.

Introduction

Available literature indicates the prognosis of patients suffering from malignant gliomas to be linked to the completeness of tumour removal [7, 1, 10, 15]. However, the surgeon's endeavour to achieve the most radical possible degree of tumour resection is often confounded by his inability to distinguish vital tumour margins from surrounding tissue under conventional white light microscope illumination. We have recently introduced a novel approach for intra-operative detection and resection of residual glioma tissue by virtue of fluorescent porphyrins, which appear to be selectively synthesized in malignant glioma tissue after administration of 20 mg 5-aminolevulinic acid per kg body weight [12]. 5-aminolevulnic acid (5-ALA), the metabolic precursor of haem in the haem biosynthesis pathway, induces the synthesis of protoporphyrin IX (PpIX) and other porphyrins with fluorescing and photosensitizing properties not only in malignant gliomas but also in malignancies of other organ systems [2, 3, 9, 6, 13, 4, 5].

We now present a more detailed description of the necessary modifications to components of the neurosurgical microscope for fluorescence-guided, microsurgical resection of malignant gliomas.

Technical Procedure

Preliminary Experience and Construction Aims

During initial attempts at fluorescence detection, the patient's tumour cavity was illuminated by a manually held liquid light guide (Karl Storz, Tuttlingen, Germany) connected to an incoherent light system emitting blue-violet light (wavelength 375–400 nm, D-Light first prototype, Karl Storz, Tuttlingen, Germany). PpIX fluorescence with characteristic peaks at 635 and 704 nm [8, 6, 2] was viewed through a 455 nm

long pass filter (OG 455, Schott, Mainz, Germany) which was likewise manually held. Although fluorescence was readily distinguished, this method required interrupting the operation with removal of the surgical microscope for assessment of tumour fluorescence, with resection possible only when the microscope was brought back into place. On the other hand, the yield of tumour fluorescence was high, because the available violet-blue illumination intensity was strong when the end of the liquid light guide was brought close (i.e. 5 cm) to the tissue surface. Powermeter measurements (Labmaster powermeter with LM10 Thermopile, Coherent, Dieburg, Germany) disclosed an irradiance of approximately 40 mW/cm^2 at a distance of 5 cm from the tip of the liquid light guide.

From this preliminary experience it was considered a basic requirement of the detection system for fluorescence-guided resection to enable switching from white to violet-blue illumination directly under the microscope – without interrupting the operation. The excitation light irradiance of 40 mW/cm^2, which was available in our preliminary attempts at fluorescence-guided resection, had been found to yield a fluorescence signal sufficient for unequivocal identification of tumour porphyrins. Basically however, it was the aim to achieve the highest level of excitation light irradiance possible at a working and illumination distance of approximately 250 mm, which is a practical working distance for surgery. Finally, it was also considered necessary to adapt a camera to the microscope, which was capable of generating a video image from the low-intensity intra-operative tissue fluorescence.

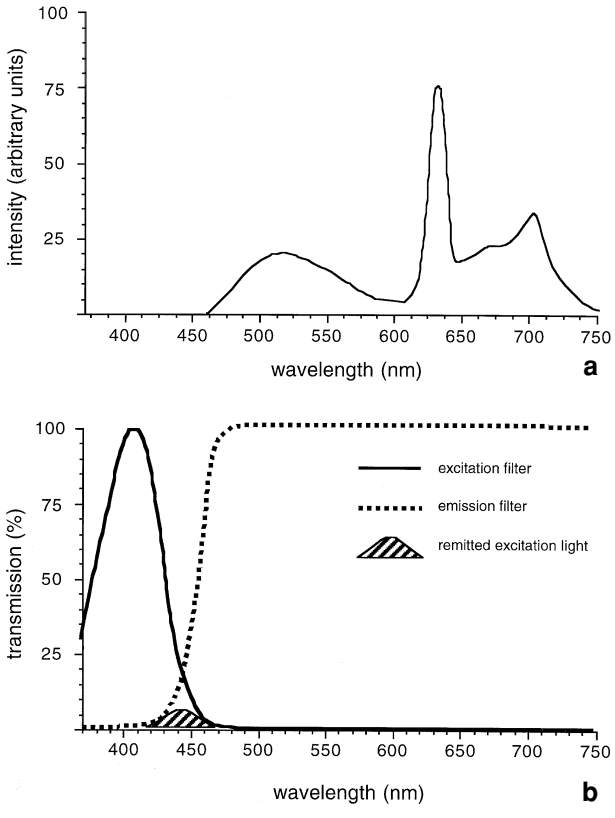

Fig. 1. (a) Fluorescence spectrum of malignant glioma with peak at 520 nm, superimposed with protoporphyrin IX spectrum demonstrating characteristic peaks at 635 and 704 nm. (b) Schematic representation of transmission spectra of excitation and observation filters. Overlap allows transmission of remitted excitation light for delineation of non-fluorescent tissue

5-ALA Induced PpIX Fluorescence Imaging

Like all porphyrins, PpIX has a strong absorption band in the violet spectral range (380–420 nm, Soret band). In tissue, the red fluorescence bands emitted by porphyrins at 635 and 704 nm are superimposed by a broad band of endogenous fluorescence (autofluorescence), peaking at approximately 520 nm (Fig. 1).

Tissue optical properties (scattering and absorption), as well as observation geometry (distance, angle, shading) influence the intensity of tissue fluorescence. This influence is dependent on wavelength. Choosing the observation filter to transmit only a single wavelength, for instance the 635 nm band ("monochrome imaging"), will thus only provide a limited impression of the real fluorescence signal, because information on tissue optical properties or observation geometry are missing in the monochrome signal. Furthermore, autofluorescence emitted from tissue also contains a faint red component, so that monochrome imaging will not allow discrimination of prophyrin fluorescence from autofluorescence. Furthermore, monochrome imaging has the distinct disadvantage of not providing tissue detail. The recognition of tissue detail is a prerequisite for conducting surgery under fluorescence detection conditions.

The drawbacks of monochrome imaging may be reduced by including additional bands of light in the emission spectrum ("multicolor imaging"). Ideally, these bands should not interfere with the fluorescence signal, while giving information on tissue optical properties, and providing tissue detail. Stepp *et al.* (1995) have demonstrated that the diffuse backscattering (remission) of blue excitation light at 445 nm follows the same dependence on tissue absorption as red porphyrin fluorescence. Thus, tissue areas with in-

creased absorption disclose fainter porphyrin fluorescence but also reduced remission of blue excitation light. Furthermore, the contrast between remitted blue light and prophyrin fluorescence is independent of geometrical factors. Showing both colours in the same image, therefore, should largely eliminate the artefactual interpretation of image information based on fluorescence intensity alone.

In addition, orientation in tissue areas void of porphyrin fluorescence should be preserved, thus forming the basis for microsurgical resection under violet-blue illumination.

The technical implementation of this concept required appropriate filtering of excitation light as well as the fluorescence signal in the observer light path. In order to enable combined perception of violet-blue excitation light together with porphyrin fluorescence, the filter characteristics of the excitation filter in the light source and the detection filter in the surgical microscope are chosen to overlap as schematically demonstrated in Fig. 1. Thus a very small part of the remitted excitation light is transmitted to the observer together with tissue autofluorescence (green) and porphyrin fluorescence (red).

Light Source and Light Delivery

The *commercially available*, incoherent light system (D-Light, 2013220, Karl Storz, Tuttlingen, Germany) is equipped with a 300 W short-arc Xe-lamp and a specifically designed dielectric short pass filter (440 nm). The short pass filter is mounted on a filter wheel and is introduced into the light path by activation of a foot pedal. The blocking factor of the excitation filter is great enough ($<10^{-4}$) to enable spectral measurements. During white light illumination an attenuator reduces irradiance to approximately 50%. Furthermore, a long pass filter (440 nm) is used to eliminate ultra-violet and violet wavelengths from the white light spectrum. This measure helps avoiding excitation of PpIX molecules in the Soret band with consequent bleaching of tissue porphyrins during surgical resection under white light. As with most organic fluorochromes, PpIX-fluorescence may be bleached by prolonged light exposure [12].

For optimum light delivery, a commercially available liquid light guide with 4.8 mm diameter and a length of 2.5 m is used (495 FR, Karl Storz, Tuttlingen, Germany). The light guide is coupled to the normal light inlet of the surgical microscope.

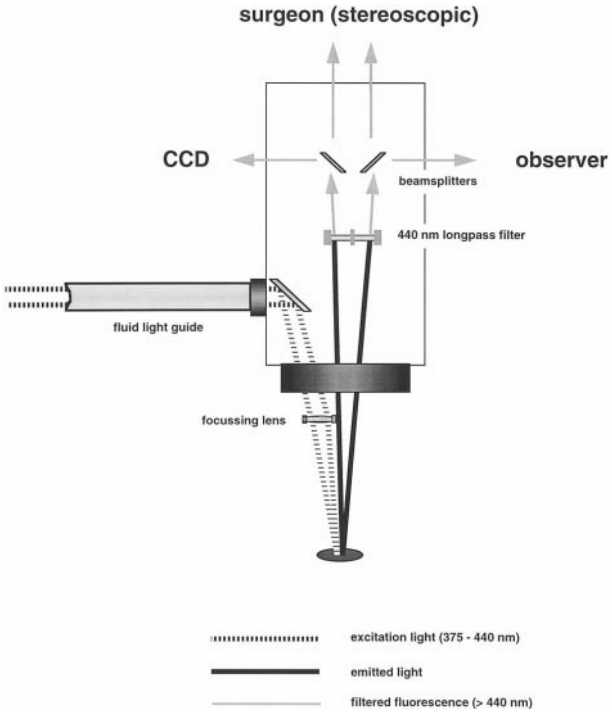

Fig. 2. Scheme depicting light pathways and filters used during fluorescence detection of malignant gliomas with 5-ALA. Xenon light filtered to blue-violet (375–440 nm) is used for excitation. A focussing lens can be introduced for greater excitation light intensity. Emitted light containing porphyrin IX fluorescence combined with excitation light is filtered in the observer light path to allow passage of fluorescence and a small fraction of (remitted) excitation light. Beamsplitters supply surgeon as well as observer and modified CCD camera

Microscope Modifications

A Zeiss OPMI CS-NC with a modified varioscope (Carl Zeiss, Oberkochen, Germany) for fluorescence observation is utilised, as schematically depicted in Fig. 2. With this varioscope, an optimized optical system is employed which increases irradiance in the center of the illumination field when using blue excitation light, but slightly reduces illumination field diameter and homogeneity. A typical value for violet blue light irradiance measured at 250 mm working length was 40 mW/cm^2 (Labmaster powermeter with LM10 Thermopile, Coherent, Dieburg, Germany).

For fluorescence observation, all apertures in the light path are opened. A laser filter (blocking infrared light exceeding 1064 nm) is left in place. Observation filters are dielectrically coated as longpass filters (440 nm; Karl Storz, Tuttlingen, Germany). The combination of excitation and observation filters enables transmission of part of the blue excitation light re-

mitted from the tissue to be perceived by the observer. We used an electromagnetic filter switcher to introduce the observation filter into the two observer light paths of the stereo-microscope. Conceivably, the longpass filters could also be fitted to any commercialy available, modular filter element for surgical microscopes. Standard beamsplitters in both light paths supply a second observer and the video camera, respectively. Thus, the surgeon is exposed to 50% of the fluorescence signal, whereas camera and co-observer each receive 50% of total emission light. The video camera is coupled to the microscope via a 65 mm adjustable focal length C-mount adapter with variable aperture.

Video Imaging

Fluorescence-guided resection in the neurosurgical operating room requires real-time video monitoring facilities. For this purpose, the camera outlet of the microscope is equipped with a 3-chip colour CCD-camera (Tricam SL PDD PAL prototype, Karl Storz, Tuttlingen, Germany) optimized for red porphyrin fluorescence detection by enhanced sensitivity in the wavelength range beyond 600 nm. The camera circuitry enables automatic or manually adjustable target integration of the porphyrin signal with exposure periods ranging from $1/10000^{th}$ of a second to 2 seconds. Typical exposure times are $1/15^{th}$ to $1/30^{th}$ of a second. Furthermore, the camera is equipped with two independent white balances for white light and fluorescence observation. When switching to the fluorescence mode, the gain of the red channel is automatically increased relative to the other channels (blue and green), in order to increase red sensitivity.

Clinical Procedure

As previously described [12], patients with suspected malignant gliomas are maintained on a standard medication of 3×4 mg of dexamethasone for a at least 3 days prior to the procedure. Three hours before induction of anaesthesia, patients received 20 mg/kg 5-ALA (Medac, Hamburg, Germany) dissolved in 100 ml of water orally. The operation commences in the usual fashion with resection of tumour portions which are easily identified under conventional white light illumination, such as areas of necrosis. When residual tumour is no longer confidently distinguished from normal brain tissue, a basic level of haemostasis is accomplished and the illumination switched to violet-blue light with simultaneous toggling of the observation filter. Room lights are dimmed. Resection is continued either under white or violet-blue light, or by switching from one illumination mode to the other.

Intraoperative Observations and Discussion

With the technical modifications described above, intra-operative fluorescence detection during surgical resection of malignant glioma was possible without disturbance of the routine procedure. Vital tumour regions were easily discerned by their vivid red PpIX fluorescence. Importantly, the border between normal tissue, which appeared bluish-grey under violet-blue illumination, and vital tumour tissue was easily recognized. Conversly, grossly necrotic tumour accumulated little to no PpIX fluorescence, which was not considered a drawback, because necrotic tumour could easily be distinguished under conventional illumination. The intensity of the red porphyrin fluorescence and accompanying, remitted blue excitation light proved strong enough for unequivocal detection of tumour fluorescence, at the same time preserving tissue detail sufficiently for extended parts of the procedure to be performed under violet-blue illumination. On the other hand, blood was found to completely absorb excitation light while lacking porphyrin fluorescence and was only recognized when it obscured light emanating from tissue. Thus, obtaining haemostasis was only possible under white illumination. Toggling from one illumination mode to the other required no mentionable periods of accommodation and did not interfere with the operation. Dimming room lights helped in distinguishing tissue porphyrin fluorescence, because ambient light in the tumour cavity was observed to interfere with the fluorescence signal.

The usage of the monopolar cautery loop for resection of fluorescent tissue was found to destroy superficial tissue fluorescence, which could easily be restored by sucking away the layer of cauterized tissue debris. PpIX fluorescence was not influenced by the ultrasound aspirator (Sonoca, Söring GmbH, Quickborn, Germany), by applying suction to tissue or by rinsing tissue with saline.

For fluorescence-guided resection of tumour, the video monitoring system was an asset but not absolutely essential for confident identification of tumour fluorescence. It was definitely required for video documentation and providing a video image to outside observers. At times, video imaging proved helpful

for interpreting the sometimes indistinct fluorescence emitted from low density infiltrating tumour regions distant to the main tumour. Under these circumstances, the camera provided a more dynamic colour contrast than available to the eye. However, care had to be taken to avoid overexposure, as nonlinearities of the video signal were found to produce a faint reddish discolouration in tissue regions devoid of PpIX fluorescence. This phenomenon was not observed with exposure times of less than $1/8^{th}$ of a second.

In our series of over 50 patients, no obvious side effects related to the oral administration of 5-ALA were noted, except for transient but slight elevation of liver enzymes which caused no discomfort to patients (unpublished data). This observation was in accordance with others [14], who describe only minor side effects after oral ingestion of higher doses of 5-ALA (30–60 mg/kg body weight). The regular histological assessment of fluorescent tissue biopsies taken from the transition zone between fluorescent and non-fluorescent tissue has not yet revealed biopsies of falsely labelled normal tissue (unpublished data).

Conclusions

The presently described arrangement and modifications to light source, surgical microscope and video camera have provided a useful tool for fluorescence-guided microsurgical resection of malignant gliomas, without disturbing the normal course of the operation. Specifically, the addition of blue remission light to the emission spectrum enabled the recognition of tissue detail to an extent that microsurgical tumour resection could continue without returning to conventional illumination.

Commercially available excitation light source, light guide and filter components allow a rudimentary form of fluorescence detection without relying on the surgical microscope. However, integration of these or similar components by microscope manufacturers should give way to an affordable system for routine and widespread utilisation of 5-ALA-induced porphyrin fluorescence as an instrument for fluorescence-guided resection of malignant gliomas.

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Comments

The authors should be commended for their report on "Technical principles for protoporphyrin-IX-fluorescence guided microsurgical resection of malignant glioma tissue". This treatment represents a significant advance in achieving complete resection of gliomas. The modification of the operating microscope to enable microsurgical fluorescence guided, more radical, tumour removal will be very welcome in neurosurgical daily practice. The preoperative preparation of the patient with peroral administration of 5-aminolevulinic acid (5-ALA) has no side effects according to the authors' preliminary studies. Technical aspects of fluorescence are described in detail regarding the physics of the light source and delivery of light as well as modifications of the optics of the microscope. The preparation of the patient is very easy, and in the neurosurgical endeavour to be more radical in resecting a glioma, it is believed that it will be accepted by many neurosurgeons and will become routinely used.

V. Dolenc

The authors describe the technical details for a new method which has recently been presented in Neurosurgery. Their article is a useful addition to this paper.

The authors present in detail the arrangement of filters and light sources. They describe their clinical experience which is safe and appears to have a great degree of accuracy. Specifically interesting is the provision of remission light to achieve lighting which at the same time provides a reliable fluorescent guidance but also allows for safe recognition of the tissue texture for regular microsurgical tumour removal. This integration appears particularly useful as in the author's description it eliminates the need to switch back and forth between different light sources.

M. Westphal

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