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mTHPC-mediated Photodynamic Diagnosis of Malignant Brain Tumors¶

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ABSTRACT

Radical tumor resection is the basis for the prolonged survival of patients suffering from malignant brain tumors such as glioblastoma multiforme. We have carried out a phase-II study involving 22 patients with malignant brain tumors to assess the feasibility and the effectiveness of the combination of intraoperative photodynamic diagnosis and fluorescence-guided resection (FGR) mediated by the second-generation photosensitizer meta-tetrahydroxyphenylchlorin (mTHPC). In addition, intraoperative photodynamic therapy (PDT) was performed. Several commercially available fluorescence diagnostic systems were investigated for their applicability in clinical practice. We have adapted and optimized a diagnostic system that includes a surgical microscope, an excitation light source (filtered to 370–440 nm), a video camera detection system and a spectrometer for clear identification of the mTHPC fluorescence emission at 652 nm. Especially in regions of faint fluorescence, it turned out to be essential to maximize the spectral information by optimizing and matching the spectral properties of all components, such as excitation source, camera and color filters. To sum up, on the basis of 138 tissue samples derived from 22 tumor specimens, we have been able to achieve a sensitivity of 87.9% and a specificity of 95.7%. This study demonstrates that mTHPC-mediated intraoperative FGR followed by PDT is a highly promising concept in improving the radicality of tumor resection combined with a therapeutic approach.

INTRODUCTION

Malignant brain tumors have an incidence of 4–10/100,000 in the European population with an increase of up to 70/100,000 in the elderly population older than 65 years. The natural life expectancy of malignant gliomas is about 3 months after diagnosis. Current treatment regimes, such as surgery, chemotherapy and radiotherapy, prolong the life span to a median survival of 15 months. Gliomas grow diffusely into normal brain parenchyma, which makes the differentiation between normal and tumorous tissue difficult or almost impossible. However, radical resection is the basis for adjunctive therapeutic modalities and corresponds directly to prolonged survival (1–5).

For the treatment of infiltrating brain tumors photodynamic therapy (PDT)† is under intensive clinical investigation because of its potential for higher therapeutic selectivity than is the case in chemo- or radiotherapy (6–8). Various exo- or endogenous dyes are currently used as photosensitizers for PDT of tumors (9); they are characterized by their tumor-selective concentration, their tumor-selective retention or both, and by their fluorescence emission under suitable excitation conditions. Recently, intraoperative photodynamic diagnosis (PDD) and fluorescence-guided tumor resection were reported by our group as well as by others (10–13).

mTHPC has already been used as an exogenous photoactive agent for PDT in a wide field of cancer treatments (14–18). In addition to the specific photophysical properties (high phototoxicity at low activation energies, high concentration ratios), mTHPC also shows a strong fluorescence. On the basis of its high affinity to neoplastic tissue, the fluorescence of mTHPC by means of blue-light excitation can be exploited for intraoperative visualization of the hardly recognizable tumor tissue, thereby essentially maximizing the extent of resection. Because of the high quantum efficiency and high sensitizer concentrations (19), the observation of the orange-pink fluorescence with the naked eye for direct fluorescence-guided resection (FGR) is also possible, despite the peak wavelength of 652 nm for mTHPC which is unfavorable for perception by the human eye.

As endoscopic diagnosis of some tumors (superficial bladder tumors, tumors of the tracheobronchial tree or oral cavity) by detection of blue-light induced protoporphyrin (PpIX) fluorescence after topical application of δ-aminolevulinic acid (ALA) has become a well-established method in the last few years (20–22), the necessary equipment is now commercially available: diagnostic systems from Karl Storz GmbH (Tuttlingen, Germany) and Richard Wolf GmbH (Knittlingen, Germany). It consists of a high-power Xe-light source (switchable from white to blue light), suitable light guides and an adapted charge-coupled device (CCD) camera with a matched observation filter. These systems are optimized for excitation and

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†*Abbreviations:* ALA, δ-aminolevulinic acid; CCD, charge-coupled device; FGR, fluorescence-guided resection; GBM, glioblastoma multiforme; PDD, photodynamic diagnosis; PDT, photodynamic therapy; PpIX, protoporphyrin IX; mTHPC, meta-tetrahydroxyphenylchlorin.

Table 1. Patient data

Patient no.	Age (years)	Gender	Diagnosis	Tumor localization
1	54	f	meta. mc	Cerebellum
2	34	f	GBM	Left fronto-temp
3	61	m	GBM	Right temp
4	42	m	max. ca	Left fronto-temp
5	54	m	GBM	Left fronto-temp
6	42	m	GBM	Right temp
7	55	m	GBM	Right temp-occ
8	57	m	GBM	Right temp
9	73	f	meta. bc	Right front
10	54	f	GBM	Left temp
11	37	f	GBM	Right front
12	67	m	GBM	Right front
13	65	f	GBM	Right occ
14	36	f	GBM	Left front
15	43	m	GBM	Right temp
16	53	f	GBM	Left temp-occ
17	52	m	GBM	Right temp
18	57	f	GBM	Right front
19	37	m	GBM	Right temp
20	49	m	GBM	Right temp
21	58	f	GBM	Right front
22	60	m	GBM	Right occ

*m, male; f, female; GBM, glioblastoma multiforme; meta. mc, metastasis of a mammary carcinoma; max. ca, maxillary carcinoma with cerebral infiltration; meta. bc, metastasis of a bronchial carcinoma; temp, temporal; occ, occipital; front, forntal.

detection of the PpIX fluorescence but are likewise suitable for mTHPC diagnosis because of similar absorption and emission properties of both porphyrins.

The ultimate goal of PDT and PDD should be the detection and the eradication of tumor cells using one and the same agent acting simultaneously as a fluorophore for tumor cell labeling and as a photosensitizer for tumor cell destruction. We have taken this logical step to combine PDD with subsequent PDT based on the sensitizer mTHPC in the treatment of gliomas (23). To our knowledge this is the first time that a combined PDT–PDD treatment with mTHPC has been carried out in the brain. Until now, 28 patients with primary or recurrent brain tumors have undergone a mTHPC-mediated PDT in our faculty, and in 22 cases a fluorescence diagnosis with the naked eye or with video assistance was performed.

MATERIALS AND METHODS

Patients. Since 1997, 22 patients, 12 men and 10 women, with primary and recurrent malignant brain tumors have been enrolled for fluorescence diagnosis and FGR. Enrolment was voluntary, and written consent was obtained from all patients before inclusion in this study, as defined in the protocol approved by the local ethical committee. The age of the patients was in the range from 34 to 73 years, with a mean value of 51.8 (*cf.* Table 1 for the clinical data of each patient). The mTHPC-lyophilized powder, provided by Scotia Pharmaceuticals Ltd (Guildford, UK), was dissolved in a solution of 20% ethanol, 30% polyethylene glycol 400 and 50% H_2O before utilization. Four days prior to craniotomy, patients were sensitized with a drug dose of 0.15 mg/kg intravenously and kept in semidark conditions for the first 4 days (dimmed room light, no direct sunlight).

Light source and light delivery. mTHPC has a strong absorption in the blue-wavelength range (the porphyrine-typical Soret band around 380–430 nm), which leads to strong fluorescence in the red with emission peaks at 652 and 718 nm (Fig. 1). The commercially

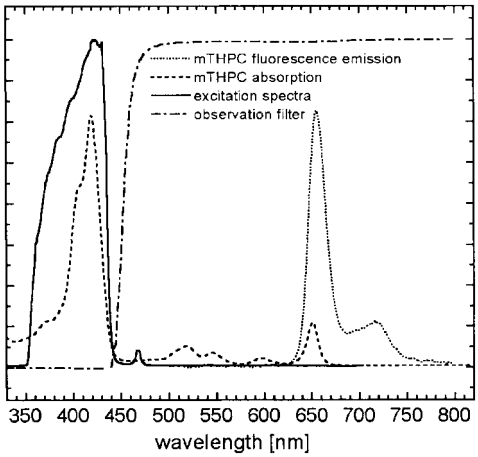

Figure 1. Spectral characteristics of the mTHPC absorption with its maximum at 417 nm (fluorescence excitation for PDD) and 652 nm (absorption peak for PDT), mTHPC fluorescence emission with a peak wavelength of 652 and 718 nm, blue excitation light from the Wolf Combilight and transmission of the observation filter GG455 (Schott).

available incoherent light sources from Storz (D-light) and Wolf (Combilight) are both equipped with a 300 W short-arc Xe-lamp and an electrically switchable filter system for white- and blue-light illumination. Because of their original usage for PpIX excitation, the broad emission of the Xe-source is filtered to match the PpIX absorption which is similar to that of mTHPC. Therefore, these light sources can also be used for mTHPC fluorescence excitation without any modifications. Both blue filter sets block the visible wavelength range very effectively, so that accurate spectral measurements in the range of 450–750 nm are practicable without disturbing the background from the excitation light source.

Normally, bundles of single glass fibers are used to deliver light to the desired application. Because of the optical losses inherent in the assembly of the fiber bundle, liquid light guides are preferable for blue-light transport owing to the achievable doubling in transmission when compared with fiber bundles. Both light sources produce a power up to 800 mW in the blue-light region (measured by Gentec power meter TPM-300, Quebec, Canada; liquid light guide with Ø = 4 mm, Wolf).

Intraoperative observation. Surgery without microscope assistance was performed as usual with resection of clearly identified tumor tissue and necrotic areas under normal white-light illumination, until differentiation of normal and tumor tissue became difficult. After thorough haemostasis, which is necessary because otherwise the blue light is completely absorbed by the blood, the resection cavity was illuminated with blue light from D-Light (Storz) or Combilight (Wolf), a liquid light guide (Ø = 4 mm, l = 3 m, Wolf) and a self-made lens system. This lens system produces a homogeneous spot (Ø = 5 cm at a working distance of 30 cm) and leads to blue-light intensities of up to 60 mW/cm². 'Suspicious' areas were examined for mTHPC fluorescence, using a hand-held filter (GG455 or GG475, Schott, Mainz, Germany) or laser-protection goggles (blocking range 200–515 nm; Laser Components, Olching, Germany). Undisturbed FGR was practicable when the goggles were used for observation. Additionally, diagnosis and documentation were performed with a video camera (Telecam, Storz or Endocam, Wolf) that was mounted on a tripod and equipped with an observation filter (GG455 and GG475, Schott). In order to increase the red sensitivity and improve the fluorescence diagnosis, the 'red' channel gain of these cameras is automatically enhanced when the light source is switched from normal to fluorescence mode. Because of generally low fluorescence intensity, fluorescence diagnosis was improved by dimming the room lights. The discrimination between fluorescent and nonfluorescent areas was based on all three detection modalities, such as the naked eye, CCD camera and spectroscopy. Biopsies were taken from fluorescent and nonfluorescent areas during the resection process. The resection was carried out until no

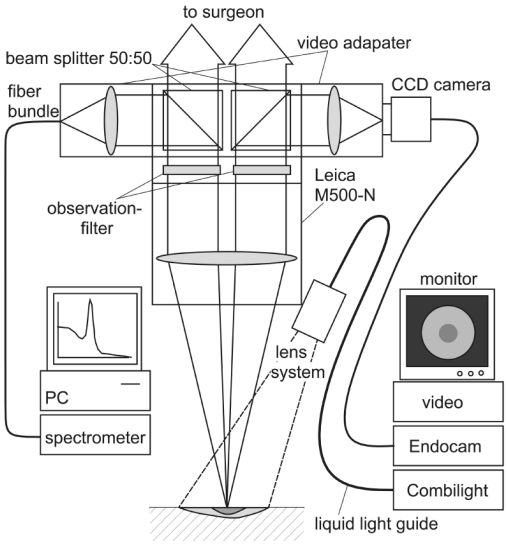

Figure 2. Modifications of the neurosurgical microscope (Leica M500-N) for mTHPC-mediated FGR and video-assisted diagnosis (Wolf). Blue excitation light is delivered by a liquid light guide to a lens system that is mounted beside the optical carrier of the microscope. An observation filter for the surgeon and the CCD camera is introduced in the optical path. Local spectral information can be obtained by a fiber-coupled sensitive spectrometer from the center part of the image.

fluorescence was visible except the fluorescence in functional areas that were left untouched. After the resection was completed to the utmost extent, biopsies were taken from the resection cavity at random.

Microscope modifications. We have modified a neurosurgical microscope (M500-N; Leica, Heerbrugg, Switzerland), on the basis of the experiences gained in our previous work, to enable PDD and FGR. Simply coupling the liquid light guide to the usual light input port of the microscope yielded a maximal blue-light intensity of 8 mW/cm^2 (light source Wolf Combilight, maximum blue-light output) which was not sufficient for clear fluorescence images. In order to enhance the blue-light intensity, a lens system similar to that used for the tumor cavity illumination was mounted beside the optical carrier of the microscope, which replaced the normal microscope illumination. The homogeneous spot permitted excitation levels of 35 mW/cm^2 at a working distance of 30 cm.

Because of the high levels of remitted blue light, fluorescence observation is only possible by blocking most of the blue light through a long-pass filter. Therefore, a stationary filter (GG455, Schott) was inserted into the optical path of the microscope (Fig. 2). The beam splitters were chosen with a splitting ratio of 50:50 to ensure sufficient light for the camera (Wolf Endocam) and the spectrometer (S & I, Erwitte, Germany) which were mounted *via* standard video zoom-adapters (Leica) with adjustable focal lengths (35–100 mm).

Color contrast enhancement. Video-assisted diagnosis is based on the (poor) color-separation ability of image sensors. Under ideal conditions the 'red' channel of the camera contains only the fluorescence information of the sensitizer, the 'green' and 'blue' channels should be built up from the tissue autofluorescence and a small part of the remitted excitation light, respectively. However, actual color-separation filters (both one- and three-chip models) have a significant spectral overlap between each color channel. Contrast, and therefore the ability to distinguish normal tissue from tumor tissue, can be improved when these crossover bands are blocked. Recently, new filters ideal for this purpose with triple band-pass design have become commercially available (XB29 Spectra^{Plus} filter, Laser Components). For the demonstration of the contrast enhancement a fluorescence phantom, similar to the one used by Wagnieres *et al.* (24), was used to simulate normal and tumor tissue fluorescence (Fig. 3). A 20 cm^3 phantom was mixed from 5 mL human albumin (20% solution; Octapharma Pharmazeutika, Austria), 5

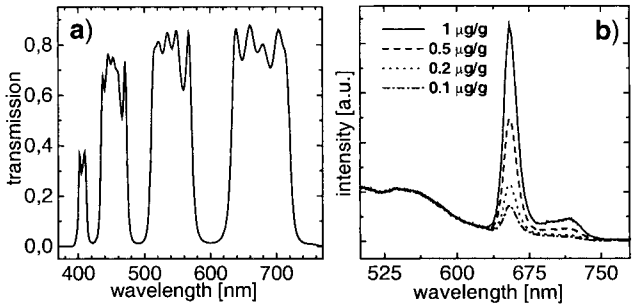

Figure 3. (a) Transmission of the triple band-pass filter XB29. (b) Emission of the fluorescence phantom with various mTHPC concentration levels after blue-light excitation (Wolf Combilight).

mL Intralipid (20% solution; Pharmacia & Upjohn, Austria) and 10 mL water and fixed in a transparent matrix of 1 g normal gelatine (Oetker, Austria). Varying amounts of mTHPC were added to simulate real tumor-sensitizer concentrations of 0.1–1 μg/g (7).

Photodynamic treatment. After intraoperative PDD and FGR, PDT was performed at a wavelength of 652 nm by a dye-laser (Laserscope, San Jose, CA) or a compact diode laser (Diomed Ltd, Cambridge, UK). The therapy light was delivered by bare fibers coupled into a modified balloon system, by a spherical distributor or by a fiber with a 20 mm long cylindrical diffusor at the distal end for interstitial treatment (Ø = 1 mm, fiber core Ø = 500 μm; Medlight, Ecublens, Switzerland). The treatment light dose was 20 J/cm^2 for superficial irradiation and 90–140 J/cm diffusor length for interstitial application.

RESULTS AND DISCUSSION

Intraoperative observation

Tumor areas with unequivocal mTHPC fluorescence could be seen in 20 out of the 22 patients by the naked eye, by video-assisted diagnosis or by both. The lack of sensitizer fluorescence in two patients (patient nos. 5 and 8) was caused by too low mTHPC concentration in the tumor tissue infiltrating normal brain parenchyma. Patient no. 17 showed no clear mTHPC fluorescence, but spectra taken with the sensitive spectrometer indicated a low but tumor-specific sensitizer concentration at certain locations. The benefit of fluorescence diagnosis was especially seen in 10 cases, where tumor tissue was revealed under blue light, which was not recognizable under normal white-light illumination after the bulk of tumor had been removed.

Both the commercially available diagnostic systems (Storz and Wolf), which were originally designed for ALA-induced PpIX fluorescence diagnosis, are suitable for mTHPC-mediated PDD. Owing to slight differences in the spectral response of the CCD cameras and the slightly different spectral characteristics of the blue-light sources as well as of the observation filters (GG475, d = 2 mm, with the Telecam and GG455, d = 1 mm, with the Endocam), the fluorescence images give rise to different color impressions: images from the Telecam exhibit some tissue autofluorescence (brownish-pink) and an orange mTHPC fluorescence, whereas images from the Endocam are dominated by the remitted blue excitation light and red-pink indications of the mTHPC presence. Despite these distinctions, areas with sensitizer fluorescence are clearly discernible in both cases (Fig. 4), especially in the available PDD modes with significantly enhanced red sensitivity.

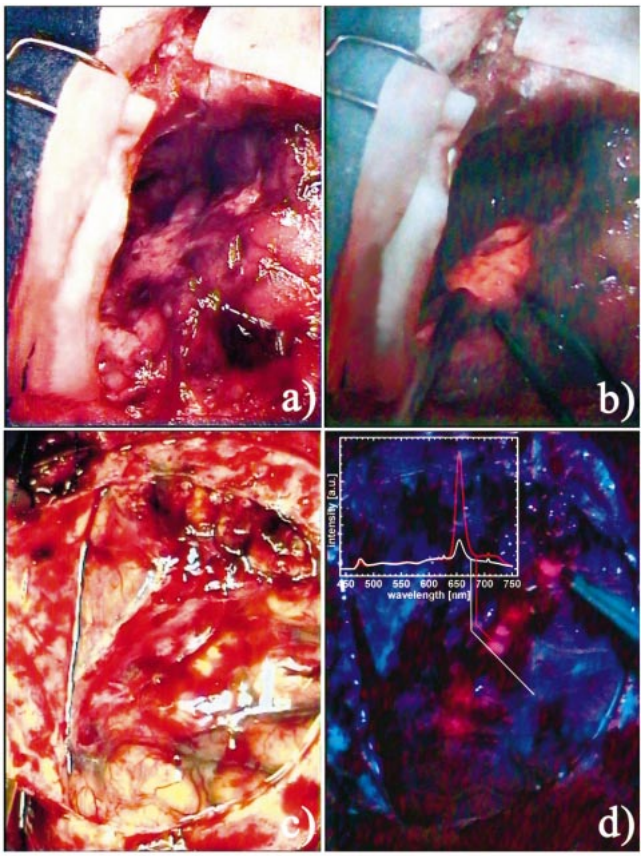

Figure 4. Intraoperative pictures under white (a, c) and blue-light (b, d) illumination of a partially resected tumor (patient 14, pictures a and b, PDD system D-Light and Telecam, Storz, camera mounted on a tripod, observation filter GG475, d = 2 mm, Schott) and the brain surface with superficial tumor portions and respective spectra (patient 15, pictures c and d, diagnosis under microscope assistance, neurosurgical microscope M500-N, Leica, PDD-system Combilight and Endocam, Wolf, observation filter GG455, d = 1 mm, Schott).

Besides serving the purpose of documentation, video-assisted fluorescence diagnosis facilitates the assessment of indistinctively low mTHPC fluorescence intensity which is hardly recognizable without any technical means because of the poor sensitivity of the human eye in this wavelength range. In contrast to enhanced PpIX bleaching during fluorescence diagnosis (12), no essential bleaching of mTHPC occurred under blue-light illumination of several minutes.

Microscope adaptions

As opposed to fluorescence diagnosis with the tripod-mounted camera, intraoperative fluorescence detection and FGR was possible during surgery without the disturbance of the routine procedure. Resection was performed under normal white-light illumination and could be changed to blue light whenever desired without interfering with the normal course of surgery. The faint yellow-colored long-pass filter (GG455, d = 1 mm, Schott) that was permanently introduced into the light path of the microscope was well tolerated by the surgeon under normal white light and preserved sufficient tissue details for resection under blue light. The small part of the blue light transmitted from the observation filter yields a very good color contrast to regions with red-pink mTHPC fluorescence (Fig. 4d). Video-based diagnosis was sometimes impaired by poor picture quality because of low fluorescence intensities. The Telecam with its possibility of target integration up to 2 s (Endocam is limited to 1/50 s) is therefore preferable for low-light applications like fluorescence imaging. In the case of weak fluorescence intensities, especially in infiltrated tissue at the tumor border (brain adjacent to tumor region), the spectral information obtained by a sensitive fiber-coupled spectrometer was beneficial. Illumination with the aid of the lens system mounted beside the optical carrier of the microscope proved to be a simple but efficient supplementation, without the need for large-scale modifications of the microscope illumination light path. This setup, however, has the disadvantage that the lateral lens system has to be readjusted depending on the working distance in order to center the spot.

Table 2. Correlation between fluorescence classification and histological assessment of tissue samples (total number of 138) obtained from brain tumor resections of 22 patients

mTHPC fluorescence	Histological findings	
	Normal	Pathological
No	45	11
Yes	2	80

Sensitivity and specificity

One hundred and thirty-eight tissue samples with intraoperative fluorescence classification (fluorescence positive or negative) were obtained during the resection of 22 patients (summarized in Table 1). Histopathological assessment revealed *Glioblastoma multiforme* (WHO grade IV) in 81 samples. Unlike the metabolism-dependent sensitizer PpIX, mTHPC is also sensitive to abnormal brain tissue. Thus, five samples with abscess-like findings, four samples of scar tissue and one necrotic sample exhibited mTHPC fluorescence and were assigned as true-positive in the earlier context of pathological findings. Correlation with the histological analysis yielded a sensitivity of 87.9%, a specificity of 95.7% and an accuracy of 90.6%. The pathologist was blind to the fluorescence classification of the tissue samples.

Contrast enhancement

Fluorescence contrast between normal and tumor tissue is essential for radical resection of brain tumors and in many other fields of optical cancer diagnosis. This color contrast depends both on the intensities of the tissue autofluorescence and the sensitizer fluorescence and on the spectral response of the detection system. Under optimal conditions, most of the fluorescence signal originates from the sensitizer, with only a small background (necessary for orientation) from the tissue autofluorescence and the remitted blue excitation light. Some of the efforts toward contrast improvement ended in the development of sophisticated and complex diagnostic systems (25–29). A new and simple way for contrast enhancement is the use of triple band-pass filters (Fig. 3) which have recently become commercially available. One major source of color contrast restriction can be identified as the

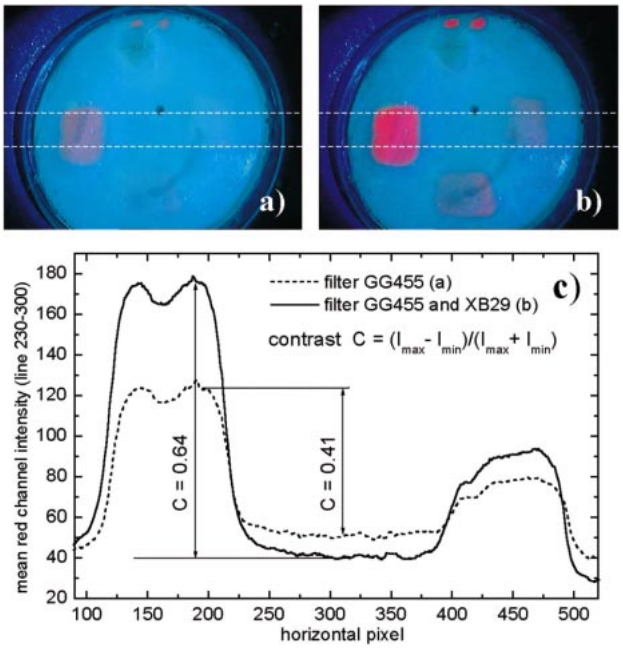

Figure 5. Demonstration of the contrast-enhancement ability of the additional triple band-pass filter XB29 by comparison of the fluorescence images of the tissue phantom (excitation with Combilight, Wolf): (a) observation filter GG455 (Schott); (b) observation filter GG455 and XB29; and (c) horizontal scan across the fluorescence images a and b (average of line 230–300, dashed white lines) indicates a 56% contrast enhancement between normal (pixel 230–370) and sensitizer (pixel 120–230 and 370–490) fluorescing areas in the red channel.

spectral cross-talk between each color channel of the image sensor. By suppressing these overlapping wavelength bands, fluorescence separation is significantly improved. The effect of the additional filter is clearly visible in Fig. 5 and indicates a contrast enhancement in the 'red' channel of almost 60% ('green' channel +160%, 'blue' channel +25%, data not shown; for definition of contrast see Fig. 5c). Because of its special transmission characteristics, the filter has a slight green coloring which can be compensated through the white balance of the video camera and is therefore not disturbing under white-light video observation. The supplementary filter combines the advantages of normal video cameras with the contrast-enhancement ability of sophisticated systems based on spectral separation, and thus offers a low-cost and straightforward means of improving the existing systems.

CONCLUSIONS

Intraoperative fluorescence diagnosis is still under intensive investigation for tumor resection using tetracycline, fluorescein and, recently, ALA and its metabolite PpIX. Whereas ALA-PDD currently represents the golden standard in clinical areas, such as urology, dermatology, pulmonology and ENT, ALA-mediated PDT is only suitable for superficial and small tumors because of the low light penetration and the higher therapeutic light dose required. The second-generation sensitizer, mTHPC, proved to be superior regarding quantum efficiency, phototoxicity and depth of light penetration, which makes it more suitable for the treatment of bulky brain tumors. Fluorescence guidance facilitates resection, significantly improving the extent of tumor removal, which might even improve survival by itself. In cases where the tumor has to be left untouched because of infiltration of functional structures, the remaining tumor tissue can be treated photodynamically. This approach of intraoperative visualization followed by intraoperative therapy is currently one of the best methods for complete eradication of malignant brain tumors.

The present study demonstrates a high degree of correlation between the presence of mTHPC fluorescence and the presence of malignant glioma, thus clearly demonstrating the great potential of mTHPC as an agent for fluorescence diagnosis and FGR, which can be summarized as ''to see and to treat''.

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