Possibility of using laser spectroscopy for the intraoperative detection of nonfluorescing brain tumors and the boundaries of brain tumor infiltrates

Technical note

SATOSHI UTSUKI, M.D., HIDEHIRO OKA, M.D., SUMITO SATO, M.D., SACHIO SUZUKI, M.D., SATORU SHIMIZU, M.D., SATOSHI TANAKA, M.D., AND KIYOTAKA FUJII, M.D.

Department of Neurosurgery, Kitasato University School of Medicine, Sagamihara, Kanagawa; and Department of Neurosurgery, Kitasato Institute Medical Center Hospital, Kitamoto, Saitama, Japan

Autofluorescence allows the identification of tumors through an assessment of uptake of the heme precursor 5-ALA, which is rapidly metabolized by tumor cells into the fluorescent molecule protoporphyrin IX. Exposure to an appropriate wavelength of light causes protoporphyrin IX to fluoresce at a 636-nm wavelength, permitting the intraoperative diagnosis of neoplastic cells by differentiating them visibly from surrounding normal cells. However, some infiltrating tumors and their marginal areas of infiltration may be subfluorescent. We therefore investigated the intraoperative use of laser spectroscopy to identify the presence of such tumors and to define the boundaries of infiltration for surgical treatment.

Clinical Material and Methods

Six patients, ranging in age from 30 to 57 years, participated in this study. None of these patients’ tumors enhanced on MR imaging following the administration of Gd–diethylenetriamine pentaacetic acid. Two hours before induction of anesthesia, each patient was given a 1-g oral dose of 5-ALA. Tumor resection was performed between 4 and 6 hours after the administration of 5-ALA. The target region was exposed to a laser light that had a peak wavelength of 405 ± 1 nm and a light output of 40 mW. The excitation light was delivered using a semiconductor laser device (VLD-V1 version 2: M & M Co., Ltd., Tokyo, Japan; Fig. 1). None of the tumors exhibited protoporphyrin IX fluorescence. The spectra of the response light from nonfluorescing tumors were analyzed. Because blood between a probe and tissue can obscure findings, histological analyses were performed on more than three samples obtained from non-bleeding areas of each tumor. Moreover, more than three samples were resected when different spectra of the response light were obtained.

Abbreviations used in this paper: 5-ALA = 5-aminolevulinic acid; MR = magnetic resonance.
Results

In three cases, protoporphyrin IX fluorescence was not observed in the target region, but a pale reflection of excitation light was visible. Spectroscopy revealed an amplitude peak at a wavelength of 636 nm (thick arrow), which is the fluorescence of protoporphyrin IX. Histological analyses were performed on samples obtained in all three cases; in two cases three samples were studied, and in one case four samples were studied. The findings confirmed the presence of tumor cells in all areas showing an amplitude peak at 636 nm, leading to a diagnosis of typical diffuse astrocytoma (Fig. 3). The specificity of these analyses was 100%. Histological analyses also were performed on samples from areas in which there had been no amplitude peak at 636 nm; in one case four samples were examined, in another five samples, and in the third case seven samples (Fig. 4). In these areas few or no tumor cells were present, from which we inferred that these sites may be areas of infiltrate. Atypical tumor cells were observed in at least seven tissue samples, and the specificity of these analyses was 44%. The area that displayed an amplitude peak at 636 nm was nearly the same as the T2 high-intensity area, and no amplitude peak at 636 nm was observed outside the T2 margin.

Discussion

The heme precursor 5-ALA is rapidly metabolized by tumor cells into the fluorescent molecule protoporphyrin IX.1,2 Exposure to an appropriate wavelength of light causes protoporphyrin IX to fluoresce, allowing the investigator to differentiate neoplastic cells from the surrounding normal cells.6 Intraoperatively, exposure to ultraviolet light causes protoporphyrin IX to fluoresce red at the 636-nm wavelength,4,10,11 permitting the diagnosis of tumors and the definition of tumor boundaries for surgical treatment. Nevertheless, some forms of tumor do not fluoresce,3,4 making a diagnosis difficult. Other tumors, particularly infiltrating tumors, may fluoresce at their centers, but their fluorescence diminishes in intensity so dramatically in regions of infiltrate that the boundaries of the tumor are impossible to observe.8 To some extent, the intensity of the fluorescence is proportional to the quantity of neoplastic cells.8 In cells with normal cytokinesis, uptake of 5-ALA is easy.7,9 However, in cases of diffuse astrocytoma, the density and division poten-

![Fig. 2. Spectrum tracing demonstrating intensity when an excitation light is applied to a tumor. A high peak is found at 405 nm (thin arrow), which is the excitation light wavelength, and a low peak is found at 636 nm (thick arrow), which is the fluorescence of protoporphyrin IX.](image1)

![Fig. 3. Light photomicrograph showing nuclear pleomorphic tumor cells. H & E, original magnification × 400.](image2)
tial of cells is not high, and thus, the uptake of 5-ALA is limited, resulting in very small amounts of protoporphyrin IX. Although this molecule is present, the amount accumulated in tumor cells is too small to produce visible fluorescence. Using our method, we were able to detect a minute amount of protoporphyrin IX at the 636-nm wavelength and were able to determine the presence of a tumor objectively. Our findings in the present study indicate that spectrum analysis surpasses fluorescence recognition in the detection of very small amounts of protoporphyrin IX. We were able to identify three diffuse astrocytomas and to delineate the boundary regions of these infiltrating tumors intraoperatively. These results suggest that spectrum analysis may provide the surgeon with an effective diagnostic tool to assess intraoperatively the definition of a nonfluorescent tumor and to demarcate the boundaries of an infiltrating tumor in cases in which the fluorescence is too faint to be observed. Because the surgical team can chemically navigate the tumor in real time, this procedure is useful for the surgical removal of a tumor including portions invading other areas. When using a conventional imaging technique, such as ultrasonography, an artifact in the form of a tumor resection stump is observed. When using the described methods, on the other hand, an artifact is not observed and we can reduce the remainder of the tumor stump cells.

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References


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Address reprint requests to: Satoshi Utuski, M.D., Department of Neurosurgery, Kitasato University School of Medicine, 1-15-1 Kitasato, Sagamihara, Kanagawa 228-8555 Japan. email: utuski@med.kitasato-u.ac.jp.