REVIEW ARTICLE

総説

**A Current Overview :
Photodynamic Diagnosis and Photodynamic Therapy using
5-Aminolevulinic Acid in Neurosurgery**

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**悪性脳腫瘍に対する光線力学的療法
—光感受性物質ALAを用いたPDDとPDTを中心に—**

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要 旨

悪性脳腫瘍の治療予後は現在の医療をもってしてもきわめて悲観的であり, glioblastomaの中間生存日数はわずかに18ヶ月でしかない。悪性脳腫瘍の治療は他臓器の癌と同様に集学的治療によって行われているが, 最近の研究で腫瘍の手術摘出率によって生命予後に大きな影響を与えることが明らかになってきた。浸潤部分も含めた腫瘍の広汎摘出によって摘出率を上げることができるが, 脳腫瘍の場合には腫瘍近傍の正常組織をも含めた広汎摘出は手術後に麻痺や言語障害などの重篤な合併症をもたらす危険性がきわめて高くなる。さらに, 悪性グリオーマは正常脳組織と肉眼的に似通っており, 手術時に区別して腫瘍組織だけを摘出する事は非常に困難である。そこで, 正常脳組織と区別して腫瘍組織だけを摘出するために肉眼で区別する方法や, あるいは運動や言語などの大切な脳機能を有している部分に腫瘍組織が浸潤している場合に, 腫瘍組織と正常組織を区別して腫瘍組織だけを治療出来る方法を究明することが緊急の課題となっている。

現在, 研究の進んでいる光線力学的医療はこれらの課題を解決する大きな手段として期待されている。光線力学的医療とは光と組織内酸素と光感受性物質の三者の光化学反応を医療に応用したものであり, 光線力学診断(PDD)と光線力学療法(PDT)を含めた総称である。

私共はすでに250例以上の悪性脳腫瘍にPDDを行い, 63例にPDTを行っている。光感受性物質は数多く開発されているが, この論文では特にALAを用いた場合を中心に解説する。

私共の経験を中心に悪性脳腫瘍に対する光線力学的医療の現在の到達点と問題点について以下のような結果を得た。1)PDDによって腫瘍摘出術中に悪性グリオーマと正常脳組織を肉眼で区別することが容易になり, 腫瘍の全摘出が可能になった。それによって生存日数の延長が期待出来るようになった。2)PDTは腫瘍が脳の深部にあって摘出困難な場合や, 運動や言語中枢部分に浸潤しているときに腫瘍組織だけを区別して治療するのに有効である。3)光線力学的医療による副作用は殆どない。4)PDDは悪性グリオーマには有効であるが良性グリオーマにはあまり有効でない。5)PDDにおいてfalse-negative, false-positive 所見を認める事がある。6)光の組織深達度に一定の限界があり大きな腫瘍塊に対してPDTの治療効果は少ない。

キーワード: PDD, PDT, ALA, PpIX, 蛍光診断, 悪性グリオーマ, グリオブラストーマ, 悪性脳腫瘍

Abstract

The treatment results for malignant brain tumors are not encouraging, and the postoperative survival time in patients with glioblastoma is only 1.5 year on an average. Although multidisciplinary treatment is conducted for malignant brain tumors similar to that for cancers in other organ, it has become apparent from recent developments in surgical methods that if a brain tumor could be completely removed macroscopically, postoperative survival time would also be significantly improved. Nonetheless, it is extremely difficult to differentiate a malignant brain tumor from normal brain tissues by naked eye or under surgical microscope. In particular, when a malignant brain tumor infiltrates into the normal brain tissues or is present near the eloquent brain area, serious complications may occur after extensive surgical removal.

Photodynamic medicine includes the use of photodynamic diagnosis (PDD) and photodynamic therapy (PDT). PDD is a diagnostic method for differentiating tumor tissues from normal tissues by detection of fluorescence in malignant brain tumor tissues during tumor removal. 5-Aminolevulinic acid (ALA) is used as a photosensitizer for PDD. PDT is a local therapeutic method for malignant brain tumors using a photosensitizer and light. Hematoporphyrin derivative (HpD) is used as a photosensitizer for PDT. In the present study, 250 brain tumor patients were treated using PDD and 63 patients underwent PDT. Based on our clinical experience, the advantages and disadvantages of PDD and PDT can be summarized as follows. Advantages: (1) PDD enabled to differentiate malignant brain tumor tissues from normal brain tissues by naked eye before tumor removal during surgery. (2) Macroscopic total removal of the malignant brain tumor was possible by using PDD, and PDD might contribute to prolong patient survival. (3) PDT might be effective for treating malignant brain tumor that has invaded the eloquent brain area and small deep-seated tumor in the brain. (4) Photodynamic medicine (PDD and PDT) has fewer side effects and offers a good quality of life. Disadvantages: (1) In PDD, it is very difficult to differentiate infiltrative tumor tissues or guerilla cells from normal tissues. Additionally, false-negative and false-positive findings are obtained, especially in brain edema tissues or recurrent tumor tissues. (2) In PDT, the depth of light penetration into the tumor tissue is limited. (3) The concentration of the photosensitizer and the oxygen in malignant brain tumor tissues is heterogeneous.

Conclusion: Photodynamic medicine involving PDD and PDT is very effective for the treatment of malignant brain tumors.

Key word : PDD, PDT, ALA, PpIX, Fluorescence Detection, Malignant Glioma, Brain Tumor

1. Introduction

Recently, dramatic advances have been made in the treatment of cancer. Early-stage cancer is almost completely treatable, and even in advanced cancer, a significant survival benefit has been achieved, depending on the tumor type.

On the other hand, the treatment of malignant brain tumors remains discouraging. The median survival time (MST) in patients with glioblastoma, which is the most malignant brain tumor, is only approximately 16 months^{5,26,31,34}.

Since the remote metastasis of malignant glioma to extracranial organs is rare, therapeutic efficacy depends on the local control of the tumor. The critical factor determining patient prognosis is histological type and grading, and other important factors affecting treatment efficacy are multidisciplinary treatments, including radical surgery, radiation therapy, anticancer drug therapy, immunotherapy and treatment of hyperthermia. In particular, it is believed that tumor resection rate effectively improves patient prognosis^{9,18,26,34}. The Committee of Brain Tumor Registry of Japan reported on the relationship between the resection rate of malignant glioma and patient survival time. The 5-year

survival rate after resecting the entire tumor was 45.8% and that after resecting 50% of the tumor was 17.5%^{32,43}. This result shows that increasing the resection rate of brain tumors during surgery prolongs the patient survival time.

Since malignant glioma invades into the normal brain tissue, this infiltrating tumor tissue coexists with the normal brain tissue. During tumor resection, it is impossible to selectively remove the malignant glioma without disturbing the normal brain tissue into which the tumor cells infiltrated. This is because it is difficult to visually differentiate the glioma tissue from the normal tissue based on the color of the former as well as to differentiate the 2 tissues based on their hardness. Moreover, extensive resection of malignant glioma along with the normal tissue is limited in order to prevent functional dysfunction of the brain. This is especially evident in the case of tumors located at the eloquent brain area, which is speech or motor functional center of the brain, wherein serious complications such as paralysis or speech disturbance occur after surgery. Due to the difficulty in the extensive resection of malignant glioma, residual malignant glioma tissue is observed despite tumor resection. To address these problems, many new

nonsurgical treatments have been under investigation. Recently, the application of photodynamic medicine has been proposed as a method to solve these problems. Photodynamic medicine includes photodynamic diagnosis (PDD) and photodynamic therapy (PDT) using a photosensitizer and light. PDD is a diagnostic method of intraoperative differentiation of the malignant glioma tissue from the normal tissue by staining with various fluorescent dyes, thereby enabling the selective removal of the former; PDT is a therapeutic method that uses a photosensitizer and light to induce necrosis or apoptosis in malignant glioma cells ¹⁾.

It is widely known that 5-aminolevulinic acid (ALA)—a heme precursor—is specifically taken up by tumor tissues and used for the biosynthesis of protoporphyrin IX (PpIX), resulting in the abundant accumulation of PpIX in tumor tissues. Navone ³³⁾ et al. reported that ALA is specifically incorporated and accumulated in breast cancer and is available for PDT. Since then, many studies have been performed on malignant tumors and ALA which is widely used as a photosensitizer in the field of photodynamic medicine ²⁴⁾.

ALA has been used as a photosensitizer in malignant brain tumors, especially malignant gliomas, for intraoperative PDD and subsequent PDT. In 1997, Stummer ³⁷⁻³⁹⁾ et al. studied the use of ALA in an experimental brain tumor animal model; subsequently, they used ALA clinically as a light-sensitive material during intraoperative PDD and PDT. Kostron ^{21,35)} and Kaneko ¹²⁻¹⁷⁾ also reported on ALA.

Intraoperative PDD using ALA for differentiating malignant glioma tissue from the normal brain tissue at the time of tumor resection and selective removal of the former without functional damage is now possible followed by an additional PDT, if residual tumor is apparent.

Trials for identifying malignant brain tumor localization by fluorescence detection after the administration of a suitable dye in patients with brain tumors are not new. Many researchers have reported these methods ever since the first attempt by Moore ³⁰⁾ et al. in 1948. However, many of these methods were not widely used in the clinical field ²²⁾. No large-scale study recruiting many patients has been performed, and few studies have reported the specific therapeutic effects of these methods. The fluorescent markers currently used for the intraoperative diagnosis of malignant brain tumors are not specifically taken up by tumor tissues; instead, their uptake is a result of the infiltration of the fluorescent markers through the blood-brain barrier (BBB), which is impaired in case of malignant brain tumors. In normal brain tissues, the fluorescent markers, when administered intravenously, cannot cross the BBB and infiltrate the tissues. This is the mechanism by which malignant brain tumor tissues can be differentiated from normal brain tissues by staining with existing fluorescent markers. A major limitation of the fluorescent markers currently used for differentiating malignant glioma tissues

from normal tissues is that the non-neoplastic edematous brain tissue located at the border between the malignant glioma and normal brain tissue can also take up these dyes. Additionally, one study has reported that the normal brain tissue possibly takes up intravenously administered dyes when it comes into contact with bleeding vessels during surgery. These are major limitations of the use of existing fluorescent markers for differentiating malignant brain tumor from the normal brain during surgery ²²⁾.

In contrast, ALA, which is an endogenous porphyrin and a precursor of heme, is naturally present in organisms and shows physicochemical properties different from those of existing fluorescent markers. ALA itself does not emit fluorescence. PpIX, which is biosynthesized in cells during mitochondrial heme synthesis from ALA, emits fluorescence upon excitation with light. PpIX is specifically accumulated in a limited number of organs and in malignant tumors. Thus, ALA has been widely used for the diagnosis of malignant tumors since fluorescence produced by PpIX upon excitation with violet-blue light is easily detected macroscopically ^{1,11,27)}.

In this report, the effectiveness of PDD with ALA during surgery, PDT using ALA, and probable issues in the future are discussed, together with the author's experience.

Local ethical committee approval was granted for these our studies and the patient's informed consent was obtained in each case.

2. Background of malignant glioma treatment

Similar to the treatment of cancers in other organs, malignant brain gliomas are treated using multidisciplinary treatments. Multidisciplinary treatments include radiation therapy, immunotherapy, hyperthermia and chemotherapy, wherein anticancer drugs are administered after extirpative surgery. However, the efficacy of these treatments is limited and unsatisfactory.

Unlike tumors in other organs, malignant gliomas are intractable, some reasons for this difference are as follows: (1) Extensive resection of the surrounding normal tissue to provide a safety margin is almost impossible since malignant gliomas are very invasive and localized in the brain parenchyma. Moreover, extensive resection is more likely to induce postoperative cerebral dysfunction. (2) A remote metastases of malignant gliomas to extradural organs is rare. (3) The existence of a specific BBB in the central nervous system that permits the passage of only a limited number of drugs. (4) Malignant gliomas show high cellular heterogeneity, which might confer varying drug sensitivity on each tumor cell.

The anticancer drug temozolomide (TMZ) which has recently been frequently used in Japan, is a landmark in the treatment of malignant gliomas ³¹⁾. The European Organization for Research and Treatment of Cancer (EORTC) ⁴⁰⁾ and National Cancer Institute of Canada performed a randomized, multicenter, phase

III clinical trial involving 573 patients with glioblastoma and compared 2 treatments, radiotherapy alone after surgery and the combination of radiotherapy and TMZ administration after surgery. The median survival time (MST) of the patients treated using radiotherapy alone and those treated using radiotherapy and TMZ combination was 12.1 and 14.6 months, respectively. The 2-year survival rates of the patients treated using radiotherapy alone and those treated using radiotherapy and TMZ combination was 10.4% and 26.5%, respectively. Thus, combination therapy significantly prolonged both the MST and 2-year survival rate. However, an MST of 14–15 months after combination therapy is not satisfactory.

Since most malignant gliomas are confined within the skull, we don't need to take the issue of metastases into consideration. Complete cure of gliomas is believed possible, if the intracranial tumor is well controlled.

Therefore, there have been many reports on operative treatment and radiotherapy, which are local therapies for malignant gliomas. Among these reports, some reports on extirpative surgery and prognosis after treatment are noteworthy.

Lacroix²³⁾ et al reported on the relationship between the extent of resection and the survival time in 416 patients with glioblastoma. A significant survival advantage was associated with the resection of 98% or more of the tumor volume (median survival, 13 months) compared with the resection of less than 98% of the tumor volume (8.8 months, $p < 0.0001$). Keles¹⁸⁾ et al also reported on the relationship between the volume of the residual tumor tissue and vital prognosis after removing the brain tumor in 107 glioblastoma patients. They reported that the MST of patients with complete disappearance of the tumor shadow, as assessed by computerized tomography (CT) or magnetic resonance imaging (MRI), was 93 weeks. Furthermore, the MST of patients with residual tumor volume of 10, 10–20, and more than 20 cc were 68.7, 49, and 50.8 weeks ($p < 0.0001$), respectively. They also observed that the volume of the residual tumor greatly influenced the time to recurrence (TTR) of the patient.

Based on these results, neurosurgeons should aim to remove more than 98% of the tumor and at least ensure that the residual tumor volume is less than 10 cc, in order to obtain the best prognosis after surgical removal in patients with malignant gliomas.

However, the peripheral regions of the tumor infiltrate the normal brain tissue since malignant gliomas are invasive tumors. If the brain area infiltrated by tumor plays an important role in brain functioning, surgical removal may induce complications such as paralysis or language disorders. Furthermore, the accurate discrimination of malignant glioma from the normal brain tissue by naked eye or under surgical microscope is difficult since malignant gliomas show the same characteristics, including color and texture, as those of the

normal brain tissue.

Thus, to achieve the removal more than 98% and the retention less than 10 cc of the tumor tissue, the real-time monitoring of the brain function of the area with the malignant glioma tissue and normal brain tissue are required. Intraoperative monitoring during PDD with ALA ensures the former, and it is possible to use PDT for the residual tumor.

Many clinical reports on PDT for malignant gliomas have been published since the PDT study in experimental animals was reported by Diamond⁶⁾ in 1972. Most of these clinical studies used hematoporphyrin derivative (HpD) as the photosensitizer for PDT. Only a few studies used ALA as the photosensitizer. Many studies have reported the complete disappearance of the tumor, as assessed by imaging examinations or the apparent prolongation of the life span of the patient after PDT. Regrettably, significant prolongation of life span after receiving PDT in patients with malignant gliomas has not been reported in a large, phase III clinical trial²²⁾.

3. PDD and PDT with ALA for malignant glioma

3.1 Porphyrin metabolism and accumulation in tumors

a) Porphyrin metabolism and accumulation in the glioma tissues

ALA is an endogenous substance, and when administered orally, it is incorporated in mitochondria, repeatedly transported between the cytoplasm and mitochondria, and finally incorporated into heme synthesis pathway. ALA is converted into porphobilinogen by the enzyme ALA dehydratase, and porphobilinogen is sequentially converted into uroporphyrinogen III, coproporphyrinogen, protoporphyrinogen IX, and finally into PpIX by protoporphyrinogen oxidase. The final product heme is synthesized from PpIX and iron (Fe) by the enzyme ferrochelatase (FeC) (Fig.1).

Harada¹⁰⁾ et al reported that PpIX shows a very strong absorbance band at approximately 405 nm wavelength (Soret band) and 4 absorbance bands between 480 and 650 nm wavelength (Q bands). That is, if tissues containing PpIX are excited using violet-blue light at a wavelength of approximately 405 nm, they exhibit intense red fluorescence bands at 635 nm wavelength.

Although normal tissues also synthesize PpIX, fluorescence is not observed macroscopically in these tissues since the amount of PpIX is very small.

After ALA administration, PpIX synthesis is observed, but PpIX accumulation is not observed in normal brain tissues. On the other hand, in malignant glioma tissues, both PpIX synthesis and accumulation are observed. It can be speculated that the rate of accumulation of PpIX changes with the differentiation from normal to malignant cells. PpIX emits red fluorescence after excitation with violet-blue light, and this fluorescence can be recognized macroscopically. Therefore, tissues containing large amounts of PpIX and showing red

Fig.1 Simplified synthetic pathway of ALA induced PpIX

fluorescence are quite likely to be tumor tissues, while those containing little PpIX and not showing red fluorescence are probably normal tissues.

Many studies have addressed the question why a large amount of the ALA metabolite PpIX is specifically incorporated and accumulated in malignant tumor cells. The following 3 hypotheses have been proposed: (1) PpIX accumulates due to the inhibition of PpIX conversion to heme in porphyrin-heme metabolism. (2) A large amount of ALA is accumulated by tumor cells due to negative feedback from the porphyrin-heme metabolic system under the condition of excess ALA. (3) ALA or PpIX itself has a high affinity to the glioma tissues.

Kondoh ^{19,20,46)}, who is one of the collaborator of the author and his colleagues, used human malignant glioma tissue removed during surgery and measured the porphyrin amount and relevant enzyme activities in porphyrin metabolism. The findings are as followings. According to their observation, after ALA administration, the amounts of all porphyrins increased in glioblastomas, which emitted strong fluorescence, as compared to those in the normal brain tissues without fluorescence. On an average, the amounts of coproporphyrin and harderoporphyrin increased by approximately 2- to 8-fold and that of PpIX increased by 8-fold (Table1). Therefore, after ALA administration, ALA dehydratase activity obviously increased and accelerated porphyrin metabolism, along with the incorporation of ALA into malignant glioma cells. It is also obvious that the material that was excessively produced and accumulated was PpIX. Furthermore, they simultaneously measured the activities of 3 enzymes involved in porphyrin metabolism, ALA dehydratase, porphobilinogen deaminase

Table1 Amount of porphyrins in the normal brain tissue and glioblastoma (ng/g wet brain)

Amount of porphyrins (ng/g wet brain) (p<0.05)

	normal brain tissue	glioblastoma	T/N ratio
PENTA	0.0	9.5	
COPRO I	16.8	46.4	2.8
COPRO III	7.6	67.0	8.8
HARDERO	23.0	134.0	5.8
PolX	125.1	1004.6	8.0

(normal brain tissue N=8, glioblastoma N=18)

(PBGD), and FeC, to investigate the mechanism underlying the increase in the amount of PpIX. They observed a trend of decrease of the activity of these enzymes with the increase in the amounts of porphyrin in malignant glioma cells. The amount of PpIX and the activity of FeC were negatively correlated.

Based on these results, Kondoh^{19,20,46)} et al proved that ALA incorporation into malignant glioma cells increased due to their aberrant growth and the activities of heme synthetic enzymes, especially of FeC, decreased with the increase in the amount of intermediate metabolites, including PpIX. These authors also speculated that FeC activity is decreased due to the activation of PBGD and presence of nitric monoxide (NO). They measured more than 30 types of trace elements in the tissues and observed a trend of iron decrease in malignant glioma tissues compared to that in the normal tissue. This suggested that heme synthesis from PpIX was inhibited due to a decrease in the concentration of iron.

A possible explanation why ALA or PpIX is oncotropic can be made by the study. Since porphyrins may have a high affinity to lipoprotein, especially to low-density lipoprotein, which is abundant in tumor cells, PpIX accumulates in tumor cells due to binding to low-density lipoproteins. However, there is no clear evidence of high affinity of PpIX to low-density lipoproteins.

b) Relationship between the BBB and ALA-induced PpIX

Hebeda¹¹⁾ et al. reported the relationship between the BBB and ALA-induced PpIX. A very small amount of PpIX was detected in the rat normal brain when ALA at 200 mg/kg was administered to a rat C6 brain tumor model.

In addition, Carcia³⁾ reported that ALA passes through the BBB. However, a clinical dose of ALA did not pass through the BBB⁴²⁾.

3.2 PDD using ALA

PDD using ALA was performed in 250 brain tumor patients at Kashiwaba Neurosurgical Hospital and Iwamizawa Municipal General Hospital by the authors. ALA is orally administered to a brain tumor patient, and red fluorescence is observed after irradiation with excitation light. The tissue showing red fluorescence is diagnosed as the tumor tissue.

a) Administration of the photosensitizer ALA

The photosensitizer ALA at 20 mg/kg concentration was dissolved in 50 mL of 5% glucose solution and administered orally to brain tumor patients at approximately 1 hour prior to the induction of anesthesia. The chemical formula and molecular weight of ALA (5-ALA, 5-aminolevulinic acid hydrochloride, Cosmo Bio Co., Ltd., Tokyo, Japan) are $C_9H_9NO_3 \cdot HCl$ and 167.59, respectively.

b) Excitation light

The violet-blue light was used as for excitation since it induces fluorescence at the wavelength of the Soret band at which PpIX shows very strong absorbance. The surgical field was illuminated with violet-blue light from a xenon lamp (Superlux 301, Carl Zeiss, Tokyo, Japan, Tokyo, Japan) through an optical fiber equipped with a filter (Nikon EX405/10, 400–410 nm, Nikon Co., Tokyo, Japan) at the distal tip. It is also possible to use other excitation light sources such as VLD-M1 (405 nm Diode Laser System, M&M Co., Tokyo, Japan) and surgical microscopes (OPMI Pentero, Carl Zeiss Tokyo, Japan) equipped with an excitation light source apparatus.

c) Macroscopic observation

The red fluorescence on excitation with violet-blue light was observed in tumor cells that contained PpIX. The surgical field showing red fluorescence is macroscopically observed. A low-cut filter (Kenko Y2, Kenko Co., Ltd., Tokyo, Japan) enables better visualization of the red fluorescence because of blockade of light of wavelengths less than 480 nm. Fluorescence can also be observed through a surgical microscope (OPMI Pentero) with a built-in excitation light and low-cut filter.

<An impressive case of practical observation in our study>

A case of recurrent glioblastoma is described here. The brain surface of the patient was exposed after incising the dura mater about 3 hours after the oral administration of ALA. The observation of the exposed brain surface with the surgical microscope (Xenon lamp, white light) revealed some lesions that were recognized due to their different color and

Fig.2 Intraoperative photograph of brain surface

- (A) Tumor surface under xenon white light. The hypervascularity is observed but the tumor boundary is vague.
 (B) By irradiating excitation light of 405 nm wavelength, red fluorescence from tumor tissues is observed and tumor boundary is clearly recognized.
 (C) Cavity after tumor removal under xenon white light, residual tumor is not clearly recognized.
 (D) Same operation field of (C) under excitation light of 405 nm wavelength, residual tumor is clearly recognized.

hypervascularity. Although this finding suggested the presence of tumor tissue, the border between the tumor tissue and normal tissue was unclear (Fig.2-A). The tumor tissue was confirmed by the apparent difference in the red fluorescence of the tumor tissue and normal brain tissue when the same lesions were excited by violet-blue light, and the tumor borders were now clearly defined (Fig.2-B). Postoperatively, no tumor shadow was observed on MRI.

Two tumor lesions, one showing very strong staining and another with weak fluorescent staining, were observed, extirpation was performed after confirming the pathology by frozen section during the surgery. Lesions showing very strong staining were tissues containing a high density of tumor cells as estimated by the frozen section. The lesions with weak staining were tissues containing a low density of tumor cells, and these lesions occasionally consisted of infiltrating tumor cells and normal cells. Necrotic lesions showed very weak or no fluorescence.

d) Fluorescence spectrum during surgery

Fluorescence difficult to be observed macroscopically can be confirmed by simultaneously measuring the fluorescence spectrum.

e) Intracellular localization of PpIX

PpIX fluorescence was confirmed in tissues with a high density of tumor cells and in the cytoplasm of tumor cells by comparing the findings of hematoxylin and eosin (HE)-stained isolated preparations and confocal laser scanning fluorescence microscopy^{8,28}.

f) Fluorescence and histopathological examination of the tumor tissue

Since tissues exhibit different intensities of fluorescence, a total of 260 biopsy specimens of both fluorescent and nonfluorescent tissue regions were obtained, and a histopathological examination was performed by HE staining in our study¹⁴. Histopathological examination revealed that tissues with very strong fluorescence represented tissues containing active tumor cells, while tissues with weak fluorescence represented a peripheral tumor infiltration area. The tissues showing blue fluorescence were normal brain tissues.

g) A representative glioblastoma case in our study for the analysis of macroscopic observation, fluorescence, spectrum, histology, and tumor volume.

A case of right temporal lobe glioblastoma in a female patient is described here. The findings of histopathological imaging, fluorescence intensity determined by naked eye, and fluorescence spectrum of the entire excised glioma in the right temporal lobe were compared. Based on these data, the tumor sizes detected by MRI, macroscopic observation, fluorescence technique, and fluorescence spectrum analysis were compared.

Fig.3 Gd enhanced MRI and photograph of a surface of resected tissue specimen

(A) Gd enhanced MRI

(B) Photograph under white light.

(C) Photograph under excitation light of 405 nm wavelength.

Fig.4 Each area which fluorescence, spectrum and histology are analyzed

Fig.5 Spectrum and R/G ratio

An MR image with gadolinium contrast is shown in Fig.3-A; a tumor is observed in the right temporal lobe.

Fig.3-B and 3-C show the cut surface of the central portion of the tumor after total resection of the right temporal lobe glioblastoma. Fig.3-B shows the cut surface under the white light; the tumor is recognized by its brownish color. Fig.3-C shows the same cut surface of Fig.3-B under excitation by violet-blue light; the fluorescence of the tumor tissue can now be recognized clearly. The maximum diameter of the tumor recognized under white light is smaller than that of the tumor recognized by red fluorescence.

The presence or absence of fluorescence as determined by macroscopic observation, fluorescence spectrum, red/green fluorescence (R/G) ratio, and histology was compared for 5 lesions, namely, A, B, C, D, and E (Fig.4). The R/G ratio is the ratio of the amplitude of the red PpIX fluorescence from the glioma tissues and the amplitude of green autofluorescence from the normal tissues in a spectrum (Fig.5). Apparent fluorescence was not macroscopically observed in lesion A (Fig.6-A). Histopathologically, lesion A comprised a necrotic tissue and

Fig.6 Fluorescence, spectrum and histology

Area A: the R/G ratio is 0.7 without a PpIX fluorescence, and pathological finding is almost necrosis.

Area B: the R/G ratio is 30.9 with an intense PpIX fluorescence, and pathological finding is an active glioblastoma. MIB-1 index is 10%.

Area C: the R/G ratio is 6.5 with PpIX fluorescence, and here is an apparent low grade glioma although a few cells have infiltrated. MIB-1 index is 5.5%.

Area D: the R/G ratio is 1.3 with PpIX fluorescence that is observed by spectrum but not macroscopy and here is an apparent low grade glioma although a few cells have infiltrated. MIB-1 index is 0.5%.

Area E: the R/G ratio is 0.6 with the absence of both macroscopic and spectrographic PpIX fluorescence in pathologically normal brain tissues.

did not show absorbance band at 635 nm that represents PpIX. Apparent strong fluorescence was macroscopically observed in lesion B. Histopathologically, this lesion showed active glioblastoma with abnormal nuclei and high cell density, and its

Fig.7 Size of tumor by different methods

A: 20 mm, B: 24 mm, C: 30 mm, D: 35 mm

MIB-1 proliferation index was 10%. In lesion B (Fig.6-B), a very strong peak at 635 nm was observed, and the R/G ratio was 30.9. In lesion C (Fig.6-C), weak fluorescence was recognized macroscopically, and most of the tumor was histopathologically identified as a low-grade glioma infiltrating into the normal brain tissue. The MIB-1 index of lesion C was 5.5%; its spectrum showed a relatively strong peak at 635 nm, and the R/G ratio was 6.5. In lesion D (Fig.6-D), no fluorescence was detected macroscopically, but its spectrum showed a very weak peak at 635 nm, and the R/G ratio was 1.3. Tissues in lesion D were histopathologically identified as normal brain tissues with few infiltrating glioma tissues (guerilla cells). The MIB-1 index of lesion D was 0.5%. In lesion E (Fig.6-E), no fluorescence was observed macroscopically, and there was no peak at 630 nm. Tissues in lesion E were histopathologically confirmed as normal brain tissues.

Fig.7 shows the size of brain tumors that can be differentiated from the normal brain tissue by 4 abovementioned measurement techniques. A tumor of 20 mm diameter could be differentiated from the normal tissue by using white light (Fig.7-A). Additionally, a tumor of 24, 30, and 35 mm diameter could be differentiated from the normal tissue by using contrast-enhanced MRI, macroscopic visualization of fluorescence by naked eye, and fluorescence spectrum, respectively (Fig.7-B, C, D). Thus, fluorescence spectrum measurement during surgery was the best method for differentiating malignant glioma from the normal brain tissue.

The largest diameter of the glioma that was distinguished from the normal tissue was 35 mm. In this lesion, fluorescence

was not observed macroscopically; its spectrum showed an obvious band at 635 nm, and it was histopathologically confirmed as glioma tissue. These results suggest that fluorescence diagnosis during surgery can successfully differentiate malignant gliomas of a size of 35 mm from the normal tissue, and fluorescence spectrum measurement during surgery can be used for diagnosis, even if fluorescence is not observed macroscopically.

h) Treatment effects of PDD

Stummer^{37,38)} et al. reported the clinical effects of PDD. The total removal rate by surgical resection of glioblastomas before using PDD was 36%. However, the total removal rate after using PDD increased to 66%. In the case of a total resection of tissues showing fluorescence, the mean survival time of patients was 101 weeks (23.5 months), whereas in case that the tissues showing fluorescence left unresected, the mean survival time was 51 weeks (11.9 months). However, this difference was not statistically significant.

And also a randomized phase III study on ALA-PDD resection of the glioblastoma by Stummer and ALA-stud group shows³⁹⁾ that median survival time (MST) of ALA removal group is 15.2 months and that of conventional removal group is 13.5 months. Unfortunately, there was not a statistically significant difference of MST between ALA removal group and conventional removal group. But, there was a statistically significant difference in the rate of recurrence at 6 months after surgery between ALA removal group and conventional removal group.

3.3 PDT with ALA

There have been few reports on the use of PDT with ALA for the treatment of patients with malignant gliomas. Generally, ALA is mainly used for the differentiation of tumor tissue from the normal tissue just prior to surgical removal of the tumor. If all tumor tissues showing fluorescence are completely removed during surgery, PDT is ineffective because the residual tumor tissue does not contain PpIX.

The author^{14,15)} and collaborator of the present study performed tumor removal based on intraoperative PDD and administered PDT to patients with malignant gliomas that were located in or near the eloquent area. In this treatment, 2 type of photosensitizers, ALA and hematoporphyrin ether (HPE) were used. Craniotomy was performed in all patients under conscious state (awake surgery), and ALA and HPE were administered before craniotomy. As much of the tumor as possible was removed under real-time monitoring, based on fluorescence diagnosis with ALA, except for the tumor in the language and motor functioning centers. PDT was performed to treat residual tumors in these eloquent areas. A total of 6 cases of malignant glioma near the motor center and motor fiber region, 2 cases of tumors near the language area, and 3 cases of intramedullary spinal cord malignant gliomas were

treated during surgery by using this method. Long-term survival of patients with glioblastoma and few complications were reported by the authors^{14,15)}.

Bisland^{2,25)} et al used PDT with ALA in a rat brain tumor model. They administered ALA to rats for several days, and PDT was simultaneously performed by photoillumination with a low dose of light for several hours. They concluded that glioma cells transplanted into the brain disappeared by apoptosis. In particular, apoptosis was selectively induced in tumor cells infiltrating into the normal brain tissue. They described this method as metronomic PDT and distinguished it from conventional PDT.

4. Toxicity and side effects of ALA

Some reports have claimed that ALA is toxic to animals and induces some side effects in humans.

The LD₅₀ of ALA was estimated to be more than 2500 mg/kg based on the data reported in single-dose studies involving oral ALA administration in rats and intraperitoneal administration in mice⁴⁾. In humans^{7,45)}, some side effects such as nausea (15%) and vomiting (5%) were observed in 42 patients with digestive tract cancer patients who were administered 30–60 mg/kg of ALA. All these side effects were observed within 2–3 h of ALA administration, and the patients recovered after several minutes. Blood tests were performed simultaneously, and no aberrant values were detected in the complete blood count (CBC), blood urea nitrogen, serum electrolytes, and creatinine measurements. Although mild abnormality of liver function in some patients was observed, it was difficult to determine whether it was caused by ALA or by the subsequent surgery. However, the liver dysfunction reportedly improved after 2 months. No episodes suggestive of acute porphyria, such as vomiting, abdominal pain, palpitation, and neuropathy, were caused by the increase in plasma ALA, although acute porphyria itself is usually a genetic disease.

As expected, photosensitivity is a matter of debate in PDT since PpIX is light sensitive. Isolation of the patient in a dark room after ALA administration is considered unnecessary since administered ALA is excreted within 48 hours and does not remain on the skin.

Regura³⁶⁾ et al. described some advantages of ALA based on their experience with ALA for fluorescence diagnosis and PDT in gastric cancer patients. These advantages are as follows: (1) Photosensitivity is not an issue because of the early excretion (24–48 h) of ALA and PpIX. (2) ALA can be administered orally, without any complications. (3) Compared to other porphyrins, ALA is highly specific to tumor cells and is not likely to be taken up by the vascular endothelium.

After oral ALA administration in 250 patients, the author and collaborator of the present study did not observe any notable side effects. However, administration of ALA to the patients with porphyria is contraindicated.

5. Future issues and perspectives

Currently, PDD with ALA is more widely used in the treatment of malignant brain tumors than PDT with ALA.

The most important aspect in the use of ALA in photodynamic medicine is that PpIX, which is induced by ALA, is not specific to malignant glioma tissues. Excessive accumulation of PpIX also occurs in other tissues. In addition to tumor tissues, PpIX accumulates also in inflammatory tissues such as acne and granulation tissue, since it depends on an abnormality in the porphyrin metabolism.

The major drawbacks of PDD are the occurrence of false-positive and false-negative results. A false-positive result is defined as the presence of fluorescence in the absence of histological confirmation of malignant glioma tissues. False positivity reflects the specificity of PDD. Conversely, a false-negative result is defined as the absence of fluorescence despite the histological confirmation of malignant glioma tissues. False negativity reflects the sensitivity of PDD. Tissues yielding false-positive results are often inflammatory cell infiltrates; thus, in case of extirpating recurrent malignant glioma, careful consideration and observation are required.

The author ¹⁴⁾ and collaborator have performed PDD in glioma patients. In these patients, the false-positive rate for malignant glioma (grades III and IV) was 2%, indicating that the specificity (true positives) of PDD was 98%. The false-negative rate was 41%, implying that the sensitivity was 59%. In benign gliomas, the reliability of fluorescence measurement was lower than that in malignant gliomas; the specificity and sensitivity were 98% and 18%, respectively ¹⁴⁾.

Utsuki ⁴⁴⁾ et al also observed false positivity. PpIX fluorescence was detected in the normal tissue in 1 of 25 cases of malignant glioma at the initial surgery, in 5 of 11 cases of recurrent malignant glioma, and in all 4 cases of metastatic brain tumors.

False positivity is generally due to an abnormality in heme metabolism after PpIX synthesis from ALA. Therefore, false-positive results are not confined to tumor tissues. If a similar abnormality is present in the inflammatory tissues, PpIX accumulates in these tissues, and fluorescence is observed.

PDD with ALA is a promising method since prior to this technique, there was no method for differentiating malignant gliomas from the normal brain tissue during the surgical removal. The surgical resection rate of malignant gliomas has dramatically increased with PDD. Stummer et al. reported that the increased surgical resection rate due to the use of the PDD using ALA has resulted in a significant longer interval until recurrence and higher survival rate at 6 months in glioblastoma patients. Further studies on the mechanism underlying the PpIX accumulation process are required, and these studies are expected to enhance the reliability of fluorescence measurement.

Studies on the use of PDT with ALA in human glioma are

at the beginning for future developments ^{11,24,27)}. There have been many reports on PDT using other photosensitizers such as temoporfin (mTHPC) ²¹⁾ and HPE ^{13,17,29,41)} in patients with malignant gliomas.

In general, the PDT response depends on a complex relationship of light, oxygen and a photosensitizer. And it is now widely understood that the extent of the tissue response to PDT is controlled by the concentration of the photosensitizer in the tissue, oxygen concentration and light dose, and that there appears to have some threshold levels for this process.

However, these methods are still unsuitable for clinical application due to problems regarding tissue penetration of light and heterogeneous distribution of light-sensitive materials and oxygen.

The technique of selective removal of malignant glioma tissue using PDD and simultaneous administration of PDT for the residual tumor seems very promising since the complete removal of even very small malignant gliomas is difficult because they are localized in the brain, which is an important organ.

A single photosensitizer, ALA, can potentially be used for both PDD and PDT.

6. Summary

The use of ALA as a photodynamic medicine yields excellent treatment results in patients with malignant gliomas. In particular, PDD during surgery enables precise discrimination of the tumor tissue from the normal brain tissue, thereby increasing the amount of tumor that can be removed. PDD also significantly prolongs the interval until tumor recurrence. Furthermore, during intraoperative PDD for identifying tumor tissues, the measurement of the fluorescent spectrum facilitates more accurate and extensive resection than that achieved with macroscopic observation of fluorescence.

Based on previous research findings, it is apparent that PpIX accumulates abundantly in malignant glioma tissues after ALA administration. Furthermore, it is evident that the mechanism underlying PpIX accumulation in malignant glioma tissues involves an abnormality in the porphyrin-heme metabolism, that is, decreased FeC enzyme activity.

Few clinical studies have been performed, and an unconfirmed treatment effect has been noted in some trials using PDT with ALA in malignant glioma patients.

ALA is advantageous because it has few complications. PDT can be administered to treat the residual tumor following tumor resection using PDD. Based on these advantages, ALA can potentially be used in the treatment of malignant gliomas.

7. Conclusion

Currently, it is essential to perform PDD with ALA during the surgical removal of malignant brain tumors, and this method increases the tumor resection rate. More studies on

PDT with ALA are expected in the future.

Nonetheless, multidisciplinary treatment, including radiotherapy and anticancer drug therapy, will continue to be important treatment strategies for malignant glioma, even if the PDD/PDT with ALA method is established.

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