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ORIGINAL ARTICLE

First autopsy analysis of the efficacy of intra-operative additional photodynamic therapy for patients with glioblastoma

Jiro Akimoto[1,2] · Shinjiro Fukami[1] · Tomohiro Suda[1] · Megumi Ichikawa[1] · Rei Haraoka[1] · Michihiro Kohno[1] · Yukiko Shishido-Hara[3] · Toshitaka Nagao[3] · Masahiko Kuroda[4]

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Abstract

The study aim to demonstrate the therapeutic tissue depth of photodynamic therapy (PDT) using the photosensitizer talaporfin sodium and semiconductor laser for malignant glioma from an autopsy finding. Three patients diagnosed with glioblastoma by pre-operative imaging (1 newly diagnosed patient and 2 patients with recurrence) were treated with intra-operative additional PDT and adjuvant therapy such as post-operative radiotherapy or chemotherapy. All three patients died of brain stem dysfunction owing to cerebrospinal fluid dissemination or direct invasion of the tumor cells from 13, 18, or 20 months after PDT. Antemortem magnetic resonance images demonstrated no tumor recurrence in the site of PDT, and autopsy was performed for the pathological analysis. Macroscopic observation demonstrated no tumor recurrence in two patients, but one patient demonstrated tumor recurrence in the therapeutic depth of PDT. Microscopic analysis demonstrated histopathological changes reaching depths of 9, 11, and 18 mm (mean: 12.7 mm) from the surface of the cavity of tumor resection, suggesting the therapeutic tissue depth of PDT to be in this range. This region demonstrated glial scarring with infiltration of T lymphocytes and macrophages, with slight degeneration of small vessel walls. However, viable tumor tissues were observed beyond or around the therapeutic tissue depth of PDT in two patients. PDT for glioblastoma prevented early local recurrence, which suggests the possibility that activation of the immune mechanisms was involved. The therapeutic tissue depth was suggested to be 9–18 mm from the surface of the cavity of tumor resection; however, the viable tumor tissues were demonstrated beyond this therapeutic range.

Keywords Photodynamic therapy · Talaporfin sodium · Glioblastoma · Autopsy

Introduction

Photodynamic therapy (PDT) was approved to be covered by health insurance in Japan in 2013 as an intra-operative additional local treatment for invasive tumor cells after maximum safe resection of the primary malignant brain tumor [1, 2]. The main mechanism of PDT is based on the generation of cytotoxic singlet oxygen in tissues under aerobic conditions, as a result of a photochemical reaction induced by the administration of the non-toxic photosensitizer talaporfin sodium (following TPS), which selectively accumulates in tumor cells, in combination with irradiation of the tumor using an excitation semiconductor laser. The cytotoxic effects of this singlet oxygen result in selective damage to tumor cells and the preservation of the brain function [1, 2].

Because the surface of the laser-irradiated area is constant (a circle of 1.5 cm in diameter: 1.76 cm^2), all irradiated areas are calculated by the number of irradiations [1]. However, the most important clinical question is the tissue depth to which this PDT method is effective. Evaluation of the treatable volume in every patient is thought to be important for predicting therapeutic effects which are associated with subsequent recurrence and survival period of patients who received PDT.

✉ Jiro Akimoto
jiroaki@gmail.com

1 Department of Neurosurgery, Tokyo Medical University, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan

2 Department of Neurosurgery, Kohsei Chuo General Hospital, Tokyo, Japan

3 Department of Human Pathology, Tokyo Medical University, Tokyo, Japan

4 Department of Molecular Pathology, Tokyo Medical University, Tokyo, Japan

We recently encountered three patients in whom we were able to evaluate the local effects of PDT by autopsy analysis. Here, we discuss the tissue changes that occurred after PDT using TPS, particularly the effective tissue depth of PDT analyzed by histopathological evaluation.

Materials and methods

This is a retrospective study carried out on three autopsied cases of malignant glioma who received standard treatment and intra-operative additional PDT between 2014 and 2016 at our university hospital. The clinical features of each case are shown in Table 1. There were two females and one male. Their age when received PDT was 34, 55, and 56 years, and when received autopsy was 35, 57, and 57 years, respectively. The resected brain tumor specimens when received PDT were pathologically diagnosed according to the revised WHO brain tumor classification [3, 4]. The case 1 and 3 were diagnosed as *IDH*-wild-type glioblastoma, and case 2 was diagnosed as *IDH*-mutant-type glioblastoma which demonstrated the malignant transformation from anaplastic astrocytoma. The case 1 was received standard chemoradiotherapy after PDT, and case 2 and 3 were received standard chemoradiotherapy prior to PDT and additional chemoradiotherapy and bevacizumab treatment after PDT. All patients died for respiratory failure by brainstem dysfunction.

Excised brain by autopsy was fixed in buffered formalin within 24 h, and tissue specimens were routinely processed into paraffin-embedded 4-μm section. The specimen of the part of the brain treated by PDT after surgical resection were subjected to hematoxylin and eosin (H&E) and immunohistochemical staining by labeled Streptavidin–Biotin LSAB ABC kits using antibodies against GFAP (6F2, 1:400; DAKO), Ki67 (MIB1, 1:200; DAKO), CD68 (ED1, 1:100; Abcam), CD8 (EP1150Y, 1:100; Abcam), CD3 (SP7, 1:100; Abcam), CD4 (EPR19514, 1:100; Abcam), and CD20 (MS4A1, 1:200; Cosmo Bio).

Representative case 1

A 56-year-old man who had a cystic tumor in the right frontal lobe was presented with a severe headache and disorientation. We administrated talaporfin sodium as an intravenous bolus at a dose of 40 mg/m^2 24 h prior to surgery, and the patient was managed under a light-shielded condition. Gross total resection of the tumor bulk was performed, and the bottom and lateral wall of the resected cavity area were irradiated five times with a 664 nm semiconductor laser (150 mw/cm^2, 27 J/cm^2). The pathological diagnosis was glioblastoma, *IDH*-wild type. The patient underwent extended focal irradiation (60 Gy) and temozolomide (TMZ; 75 mg/m^2; Stupp regimen) for 42 days and the patient returned to work.

Table 1 Clinicopathological summary of the three patients

Case	Age at autopsy	Gender	Duration from PDT to autopsy (month)	Local radiological findings after PDT	Depth of local histological change by PDT (mm)	Remnant of the Ki67-positive tumor cell	Gliotic scar	Macrophage infiltration	T lymphocyte infiltration	B lymphocyte infiltration	Vascular changes
1	57	Male	20	Recurrence of the tumor (−)	9	Absent	Abundant	Faint	Faint	Absent	Minimum
2	35	Female	14	Recurrence of the tumor (−)	18	Faint	Abundant	Abundant	Faint	Absent	Minimum
3	57	Female	16	Recurrence of the tumor (+) closely to PDT area	11	Faint	Faint	Abundant	Abundant	Faint	Prominent

He underwent maintenance chemotherapy of 13 cycles of TMZ (150–200 mg/m^2) in the outpatient clinic, but anorexia and nausea appeared 16 months after the first surgery. Magnetic resonance imaging (MRI) displayed no apparent findings of recurrence in the primary site, but a large tumor in the fourth ventricle and cerebrospinal fluid dissemination of tumor cells were detected. Decompressive resection of the recurrent glioblastoma in the fourth ventricle was performed, but the patient died of respiratory failure 59 days after reoperation. The clinical course from the first surgery with PDT was 20 months (Fig. 1a–h).

Autopsy findings

The surface of the cavity of tumor resection and five times laser irradiation was smooth, and there were no macroscopic findings indicating tumor recurrence. H&E staining of sections including the area treated by PDT showed decreased staining with eosin to a depth of 9 mm from the surface of resected cavity. However, the region beyond this therapeutic depth demonstrated viable tumor tissue. The lesion in the therapeutic tissue depth consisted of GFAP-positive reactive gliosis with abundant infiltration of CD68-positive macrophages and few CD8/CD3-positive T lymphocyte, with few atypical tumor-like cells on the edematous matrix. In this region, vessel walls showed thinning without hyalinization. A large, (5.5 cm diameter), cerebellar gray–white colored tumor with necrosis was detected, which extended to the fourth ventricle and invaded to the thalamus and brain stem. The histological finding was glioblastoma, *IDH*-wild type (Fig. 2a–h).

Representative case 2

A 30-year-old woman had a diffuse glioma in the left frontal lobe, presented with a generalized seizure. The patient underwent gross total resection under awake craniotomy and additional extended focal radiotherapy. The pathological diagnosis was *IDH*-mutant type anaplastic astrocytoma. Thirty-seven months after the first surgery, local recurrence of the tumor was detected and total resection was performed again under awake craniotomy again. The tumor showed malignant transformation, and the histopathological diagnosis was *IDH*-mutant-type glioblastoma. Therefore, we added the maintenance chemotherapy using TMZ. However, 14 months after the second surgery, the tumor was locally recurred and invading to the corpus callosum and basal ganglia. We underwent surgery under awake craniotomy again, and added four times of PDT to the corpus callosum and basal ganglia. After the third surgery, her performance status (PS) was improved, and bevacizumab (BEV) was administered

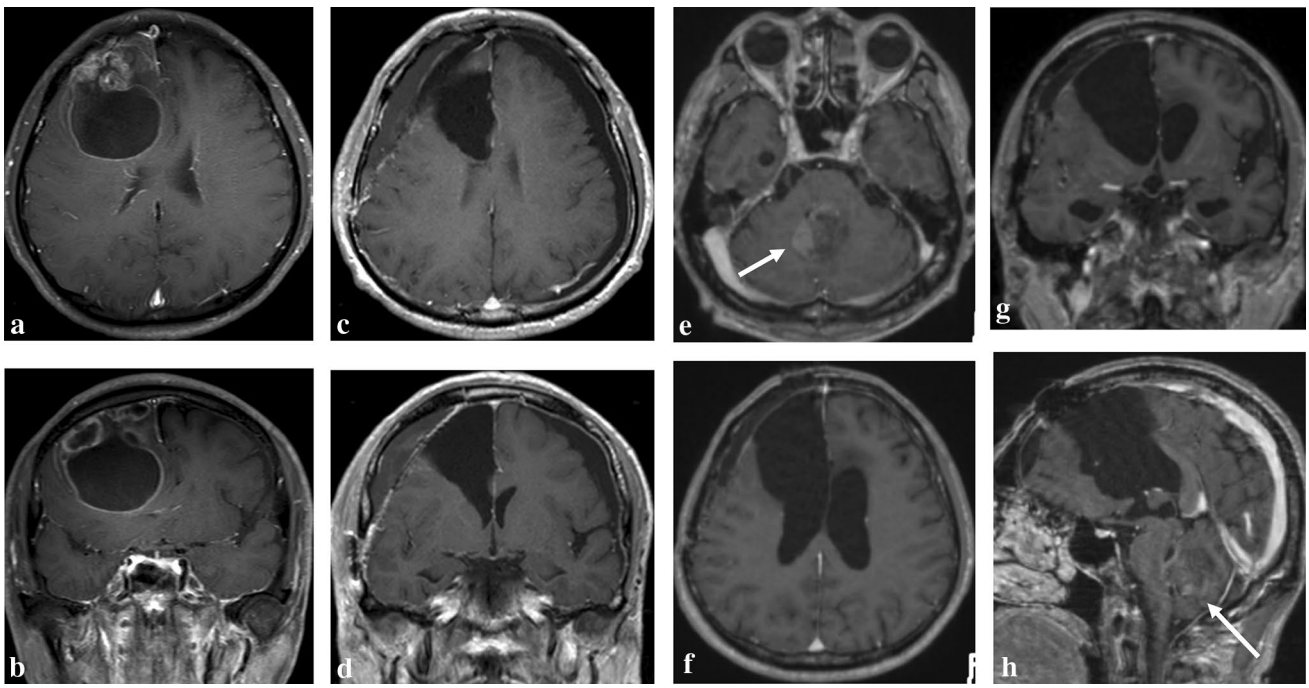

Fig. 1 Sequential MRI findings (T1-weighted images with gadolinium) of patient 1. **a**, **b** Pre-operative axial (**a**) and coronal (**b**) images displaying right frontal cystic glioblastoma. **c**, **d** Post-operative axial (**c**) and coronal (**d**) images displaying the effects of gross total resection with five times laser irradiation to the left frontal white matter. **e**–**h** 16 months after the first surgery, a tumor recurring at a distant site occupying the fourth ventricle was displayed (arrow) without any finding of local recurrence (**e**, **f** axial images, **g** coronal image, **h** sagittal image)

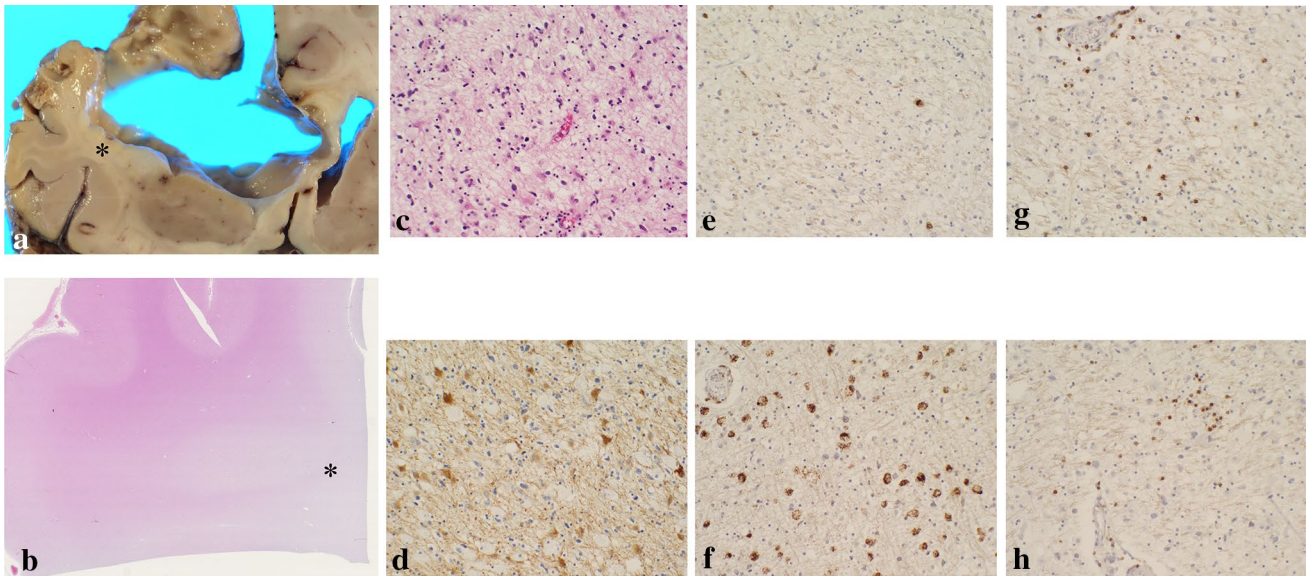

Fig. 2 Finding on autopsied brain sections of patient 1. Macroscopic findings (**a**), and microscopic findings of hematoxylin and eosin staining (**b** and **c**), and immunohistochemical analysis of glial fibrillary acidic protein (GFAP) (**d**), Ki67 (**e**), CD68 (**f**), CD8 (**g**) and CD3 (**h**). **a** Macroscopic observation demonstrated that the surface of the cavity of tumor resection was smooth, indicating the absence of prominent tumor recurrence. (asterisk: therapeutic area of PDT). **b** Staining of eosin was decreased in the therapeutic area of PDT (asterisk). The depth of the histological changes was 9 mm from the surface of the cavity of tumor resection. **c**, **d** The therapeutic area of PDT demonstrated proliferation of GFAP-positive astrocytes and infiltration of inflammatory cells in the edematous matrix (**c** ×100, **d** ×200). **E–h** The therapeutic area of PDT contained few Ki67-positive proliferating cells (**e**) and infiltration of abundant CD68-positive macrophage (**f**), with few CD8-positive (**g**) and CD3-positive (**h**) T lymphocytes (**e–h** ×200)

biweekly. However, her PS was gradually decreased, and MRI demonstrated the diffuse invasion of the tumor to the bilateral basal ganglia, brain stem, and cerebellum. The patient died of respiratory failure 14 months after the third surgery with PDT. The total clinical course from the first surgery was 65 months (Fig. 3a–j).

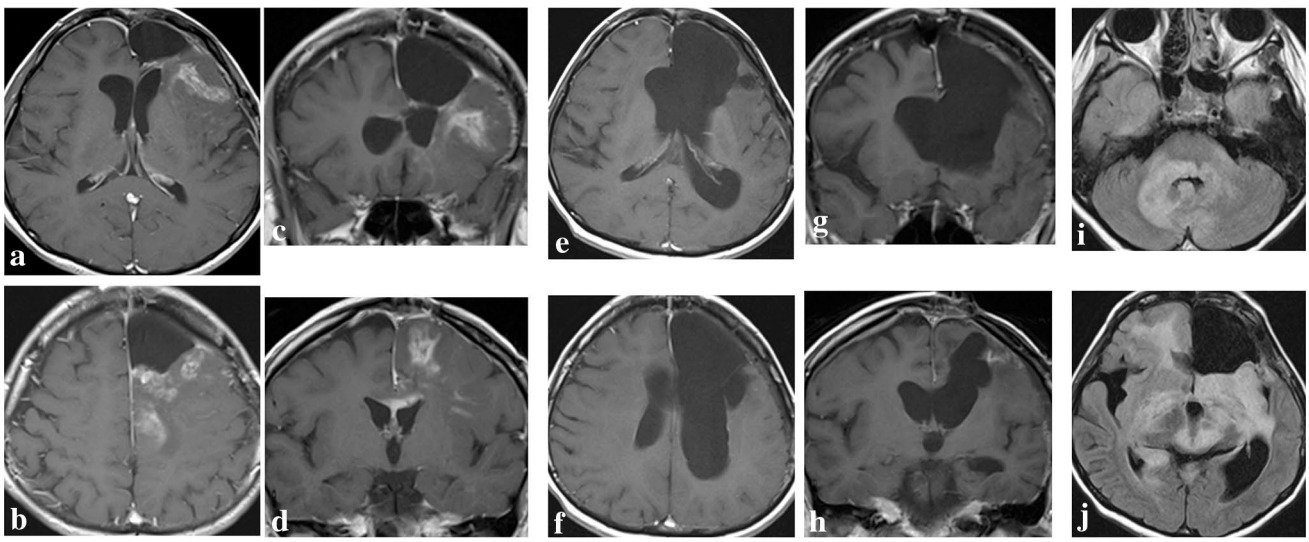

Fig. 3 Sequential MRI findings (T1-weighted images with gadolinium) of patient 2. **a–d** Pre-operative axial (**a**, **b**) and coronal (**c**, **d**) images displaying diffuse infiltration of recurrent left frontal high-grade glioma around the resected cavity before the two surgeries. **e**, **f** Post-operative axial (**e**) and coronal (**f**) images displaying the effects of gross total resection with four times laser irradiation to the left basal ganglia and corpus callosum. **g–j** 14 months after the last surgery, premortal MRI (**i** and **j** FLAIR images) displaying the diffuse infiltration of tumor tissue to the bilateral basal ganglia, brain stem, and cerebellum; however, no local recurrence was detected (**g** and **h**)

Autopsy findings

The surface of the cavity of tumor resection and four times laser irradiation of laser was smooth, and there were no macroscopic findings indicating tumor recurrence. H&E staining of sections including the area treated by PDT showed a decrease in eosin staining to a depth of 18 mm from the surface of the resected cavity. No viable tumor tissues were detected up to this therapeutic depth. The lesion in the therapeutic tissue depth demonstrated GFAP-positive reactive gliosis with abundant infiltration of CD68-positive macrophages and few CD8/CD3-positive T lymphocytes, with few atypical degenerated tumor-like cells. In this region, the vessel wall showed abundant fibrinoid degeneration. On the other hand, the brainstem, including the medulla oblongata showed abundant tumor cells invasion (Fig. 4a–j).

Representative case 3

A 44-year-old woman had a right temporal tumor that was incidentally diagnosed when she was involved in a traffic accident, and she underwent subtotal resection in another hospital. The pathological diagnosis was glioblastoma. She received boron neutron capture therapy after surgery. However, only 6 months after the surgery, local tumor recurrence was found and she underwent gross total resection and post-operative maintenance chemotherapy using TMZ. For 10 years, she received maintenance TMZ chemotherapy and the local control of the tumor was achieved, but owing to financial problems, she discontinued TMZ maintenance therapy. Thirteen months after discontinuing TMZ therapy, local tumor recurrence occurred and she was referred to our hospital. MRI displayed recurrent tumor in the right temporal lobe with invasion to the anterior part of insular cortex. The patient underwent subtotal, resection of the tumor and PDT was performed three times to the anterior part of the insular cortex. The pathological diagnosis was glioblastoma, *IDH*-wild type. After surgery, the patient underwent TMZ rechallenge; however, 7 months after the surgery with PDT, the tumor was recurred in the surface dura mater of the temporal lobe. The patient underwent gross total resection of the tumor, and the pathological diagnosis was gliosarcoma. Five months after the surgery, diffuse leptomeningeal dissemination of the tumor was detected, so she underwent intensity-modulated radiation therapy, BEV, and low-dose ifosfamide, cisplatin, and etoposide therapy. However, the patient died of respiratory failure 16 months after the third surgery with PDT (Fig. 5a–j).

Autopsy findings

The surface of the cavity of tumor resection was smooth and the area that received three times laser irradiation of laser was consisted of necrotic structures on macroscopic observation. Tumor tissues were located in the subdural space of the left frontal operculum, intraventricular and subarachnoid space of the surrounded brain stem, and invaded into the medulla oblongata. H&E staining of sections including the area treated by PDT showed decreased staining of eosin up to a depth of 11 mm from the surface of the resected cavity. The area consisted of necrotic tissue and atypical degenerated tumor cells that were Ki67 positive in the deepest area.

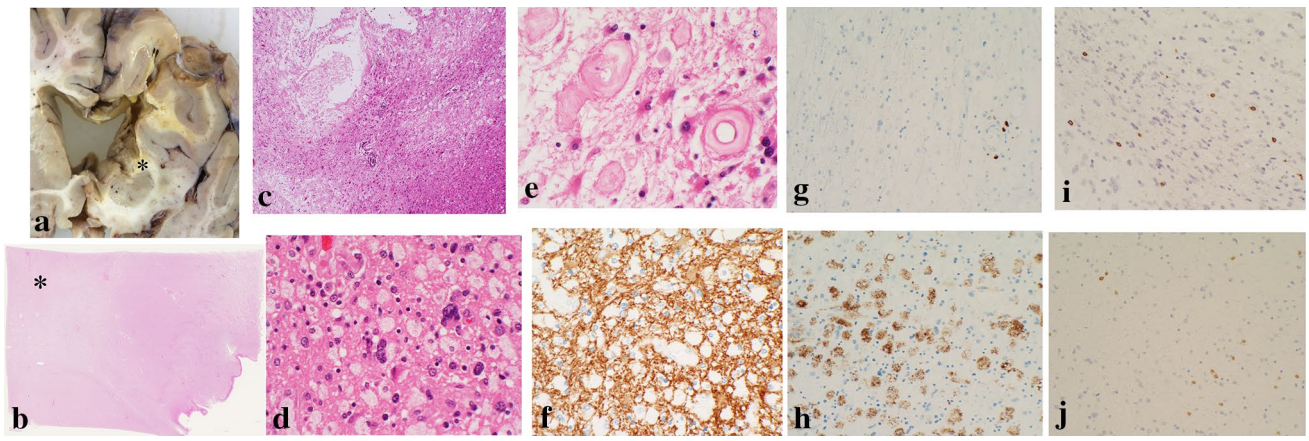

Fig. 4 Findings on autopsied brain sections of patient 2. Macroscopic finding (**a**) and microscopic findings of hematoxylin and eosin staining (**b**–**e**), and immunohistochemical staining of GFAP (**f**), Ki67 (**g**), CD68 (**h**), CD8 (**i**), and CD3 (**h**). **a** Macroscopic observation demonstrated that the surface of the cavity of tumor resection was smooth and without prominent tumor recurrence (asterisk: the area treated by PDT). **b** Staining of eosin was decreased in the area of PDT (asterisk). The depth of the histological changes was 18 mm from the surface of the cavity of tumor resection. **c**–**e** The therapeutic area of PDT consisted of proliferation of glial cells, foamy macrophages, and few atypical glial cells in the edematous matrix (**c** ×40, **d** ×200). And fibrinoid degeneration of microvessels was demonstrated (**e** ×200). **f**–**j** The therapeutic area of PDT contained abundant GFAP-positive astrocytes (**f**), few Ki67-positive proliferating cells (**g**), and infiltration of abundant CD68-positive foamy macrophages (**h**), with few CD8-positive (**i**) and CD3-positive (**j**) T lymphocytes (**f**–**j** ×200)

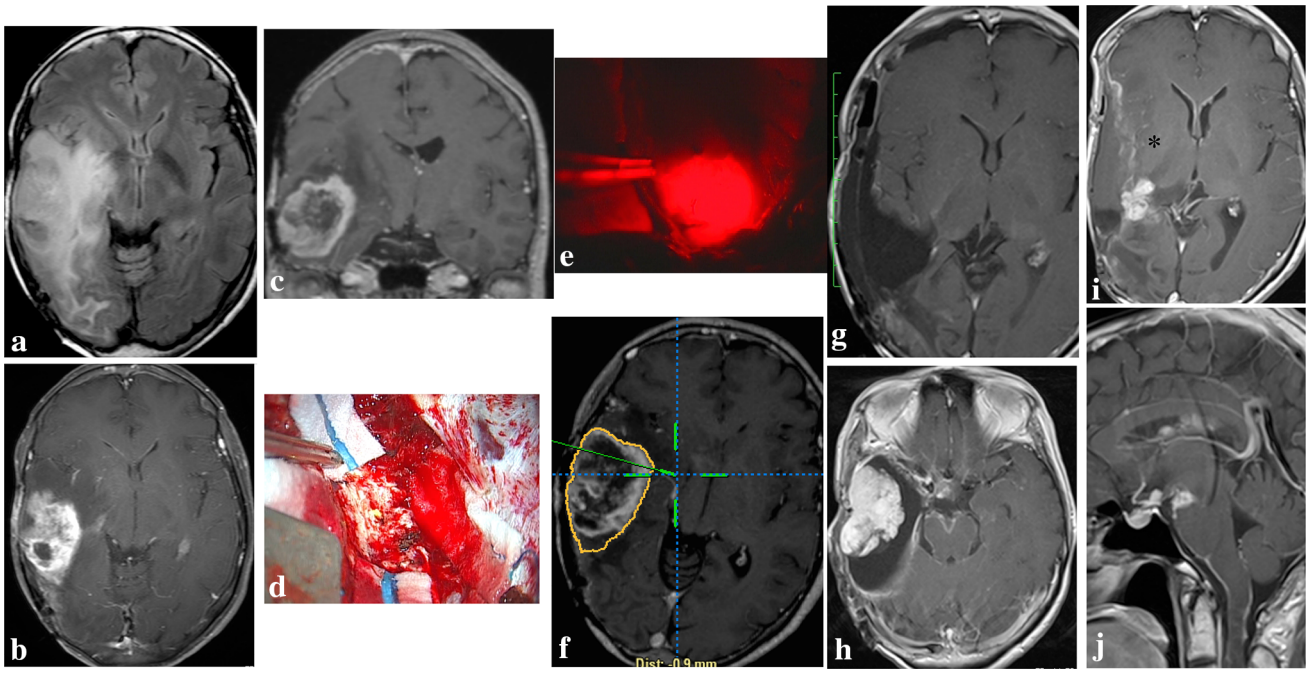

Fig. 5 Sequential MRI findings (T1-weighted images with gadolinium) of patient 3. **a**, **b** Pre-operative axial FLAIR image (**a**), axial (**b**), and coronal (**c**) section of T1-weighted imaging with gadolinium enhancement, displaying recurrent right temporal glioblastoma with extensive perifocal edema. **d**–**f** After gross total resection of the tumor bulk (**d**), laser irradiation was performed three times (**e**) and the irradiated area was confirmed on intra-operative navigation imaging (**f**). **g** Post-operative axial image displaying gross total resection of the tumor. **h** 7 months after surgery, the tumor recurred from the surface dura mater without local recurrence in the area of laser irradiation. **i**, **j** 16 months after surgery, antemortem MRI (**i** axial, **j** sagittal image) displayed a recurrence of adjacent area of PDT and cerebrospinal fluid dissemination of the tumor; however, local recurrence was not detected in the area (asterisk) treated by PDT

This area consisted of abundant small vascular structure with fibrinoid necrosis and GFAP-positive reactive astrocytes, and the accumulation of CD68-positive macrophages and CD8/CD3-positive T lymphocytes. On the other hand, the brainstem, including the medulla oblongata showed extensive aggressive tumor cells invasion (Fig. 6a–h).

Clinicopathological summary (Table 1)

We have briefly summarized the clinical course and autopsy findings of the three patients in Table 1. The timing of laser irradiation was for a newly diagnosed glioblastoma in one patient and for recurrent glioblastoma in two patients. The patient with newly diagnosed glioblastoma received radiotherapy after PDT, but both of the recurrence patients received PDT after the radiotherapy. Autopsy was performed on the patients 14–20 months after PDT, and all three patients demonstrated no prominent local recurrence in the area receiving PDT. This was not consistent with cerebrospinal fluid dissemination of the tumor on antemortem MRI. Autopsy demonstrated no recurrent viable tumor cells in the therapeutic area of laser irradiation both macroscopically and microscopically, but patients 1 and 3 demonstrated viable tumor tissues beyond this area microscopically. These areas consisted of extensive GFAP-positive glial scarring with the invasion of macrophages and T lymphocytes from the surface of the cavity of tumor resection to a depth of 9–18 mm. However, the limitations in the therapeutic depth of PDT were also demonstrated.

Discussion

Because PDT is a intra-operative local treatment used in combination with additional therapies, such as chemoradiotherapy for glioblastoma, it is difficult to evaluate the solitary effect of PDT. We speculated the possibility of evaluating the efficacy of PDT on post-operative MRI, because our method of PDT is surface irradiation to the cavity after resection of the tumor bulk. However, although some preclinical experimental report on this topic has been published, clinical reports have not been published to date [5, 6].

Singlet oxygen, which is generated by a photochemical reaction of PDT, induces not only the apoptosis and necrosis of glioma cells selectively and the shutdown of tumor neovascularization, but also the activation of the local immune system [7–10]. The radiation range of PDT is determined

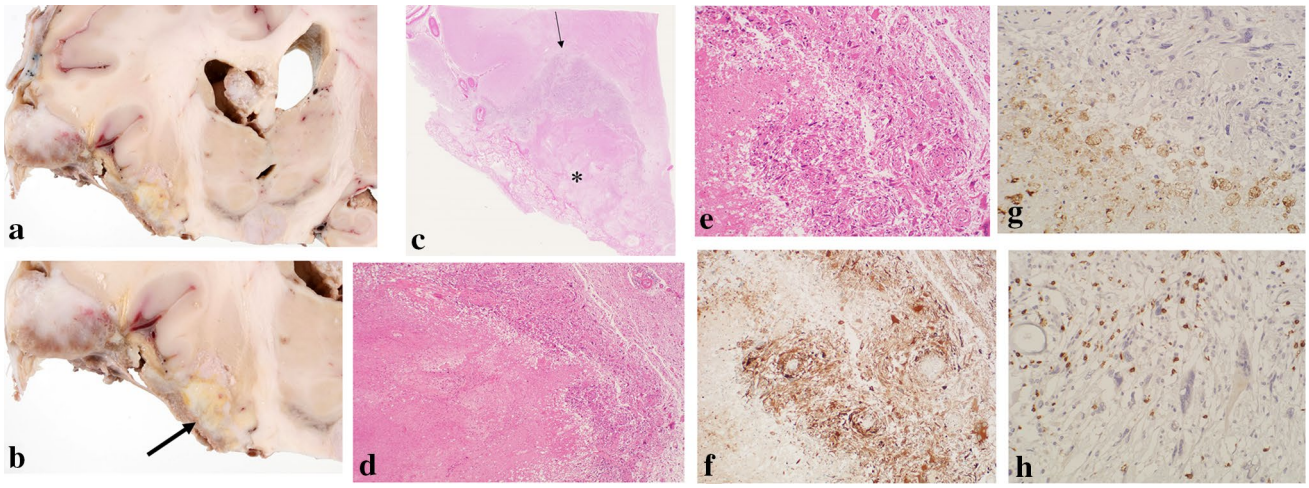

Fig. 6 Finding on autopsied brain sections of patient 3. Macroscopic findings (**a** and **b**) and microscopic findings of hematoxylin and eosin staining (**c**–**e**), and immunohistochemical analysis of GFAP (**f**), CD68 (**g**), and CD8 (**h**). **a**, **b** Macroscopic observation demonstrated that the surface of the cavity of tumor resection showed focal necrosis (arrow) surrounded by viable tumor tissue. **c** Staining of eosin was decreased in the area treated by PDT (asterisk). The depth of histological changes was 11 mm from the surface of the cavity of tumor resection. Viable tumor tissue (arrow) was surrounded by the necrotic area. (semi-macro image). **d**, **e** The therapeutic area of PDT consisting of abundant necrosis and viable tumor tissue was found to extend beyond the necrotic area (**d** ×40, **e** ×200). **f**–**h** Area between the necrotic area and viable tumor tissue consisted of abundant, GFAP-positive astrocytes (**f**), CD68-positive macrophages (**g**), and few infiltration of CD8-positive T lymphocytes (**h**) (**f**–**h** ×200)

by the surgeon, but the radiation depth depends on the wavelength of the laser beam. There are few reports to date regarding the depth of light delivery of a semiconductor laser beam of 664 nm to the brain, but some reports have speculated a penetration depth of the laser beam of approximately 4–5 mm in normal brain structures and approximately 10 mm in the edematous brain structures of the regions of tumor invasion [8]. Findings on brain autopsy of three patients demonstrated that histopathological changes of tissues in the PDT area occur in regions of 9–18 mm in depth. However, we cannot determine whether these histopathological changes were a solitary effect of PDT, as mentioned above. In addition, autopsy findings demonstrated that the viable tumor cells had not completely disappeared, and therefore, these findings indicated the therapeutic limitation of the PDT. We speculate that invasion of macrophages and T lymphocytes in brain region receiving PDT might indicate the activation of immune mechanism by PDT [9, 10]. Recently, Nitta et al. reported that 30 patients with newly diagnosed glioblastoma who underwent PDT using TPS demonstrated a significantly lower rate of local recurrence than patients not receiving PDT in their own institute [11]. We would like to emphasize that our present autopsy findings are valuable data supporting the results of this report.

There are several limitations to acknowledge in this study. First, only three patients were analyzed in this study, and they included both patients with primary tumor and recurrent tumors, and the patients varied in clinical condition, treatment regimen, and whether PDT was performed prior to radiotherapy or after radiotherapy. Second, there was a limit in describing the effect of the PDT only by the histopathological analysis on brain autopsy. However, we would like to emphasize that to our knowledge, this is the first report to date confirming the effects of PDT on glioblastoma at autopsy.

Conclusion

We reported three patients in which the effects of PDT on glioblastoma were confirmed by histopathological analysis of autopsy brain samples. We showed the glial scarring with the inflammatory cell infiltration and the disappearance of viable tumor cells at a depth of 9–18 mm from the laser irradiation surface. Antemortem neuroimages did not show local recurrence in all patients, and all patients died of brain stem dysfunction by the cerebrospinal fluid dissemination. Therefore, we were able to confirm the local control and the therapeutic limitations of PDT for glioblastoma by this first autopsy analysis.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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