Fluorescence-guided resection of glioblastoma multiforme by using high-dose fluorescein sodium

Technical note

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The extent of surgery performed has been regarded as one of the significant prognostic factors in many studies of GBMs, although there are some controversies. Cytoreductive surgery aimed at GTR apparently plays a significant role in prolonging survival, at least in some patients with GBM. The proliferation of computer-associated neuroimaging modalities has facilitated the safe and precise removal of brain tumors and postoperative evaluation of the extent of surgery. In addition, recent advances in neuronavigational systems, neurofunctional imaging, and intraoperative neurophysiological monitoring have increased the rate of radical tumor resection even in patients with GBMs. Achieving GTR of a GBM is actually difficult in some cases, however, because of the lesion’s biological properties; that is, the border between tumor tissue and normal brain tissue cannot be recognized with the naked eye during surgery because of tumor cell infiltration.

We have recently used a fluorescence-guided tumor resection procedure by using high-dose fluorescein sodium without any special surgical microscopes for the intraoperative visualization of glioblastoma multiforme (GBM), and they report on the actual procedure and clinicopathological findings.

Thirty-two patients with GBMs underwent tumor resection during which this fluorescence-guided procedure was used. Fluorescein sodium (20 mg/kg) was intravenously injected after dural opening at the craniotomy site. The tumor was stained almost homogeneously yellow and the color was intense enough to be readily perceived for resection. The center of the solid lesion was stained a deep yellow and surrounded by a transition zone that was faintly stained. The colored lesion was clearly distinguishable from the unstained zone outside the GBM, particularly in the white matter. Both the deeply and faintly stained regions included endothelial proliferation and dense tumor cells. In the unstained region, less dense tumor cells were consistently revealed; however, no endothelial proliferation could be seen. Gross-total resection (GTR) was successful in 84.4% of the patients who received an injection of fluorescein sodium, which accounted for 100% of those in whom all the visible yellow color (both the deeply and faintly stained regions) was judged to have been resected during operation. Gross-total resection was performed in 100% of the patients who underwent the fluorescence-guided procedure and assigned to Stage I, a GBM stage in which, as a therapeutic policy, the tumor should be resected as radically as possible. The GTR rates in patients who received fluorescein sodium were significantly higher than those in patients who did not (73 patients with GBMs who underwent tumor resection without the fluorescence-guided procedure). Although the extent of surgery was revealed to be one of the significant and independent prognostic factors for GBM, the fluorescein sodium–guided resection procedure was not a significant or independent prognostic factor in this series.

This surgical procedure does not require any special surgical microscopic equipment and is simple, safe, useful, readily accomplished, and universally available for resection of GBMs. Its efficacy simplifies the surgical procedure of navigating the stained lesion from the unstained area to achieve GTR of GBMs, which can be demonstrated on magnetic resonance images.

**KEY WORDS** • glioblastoma multiforme • fluorescence-guided surgery • fluorescein sodium • blood–brain barrier

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**Abbreviations used in this paper:** ACNU = (1-4-amino-2-methyl-5-pyrimidinyl)-methyl-3-(2-chloroethyl)-3-nitrosourea; BBB = blood–brain barrier; GBM = glioblastoma multiforme; GTR = gross-total resection; KPS = Karnofsky Performance Scale; MR = magnetic resonance.
After induction of general anesthesia, high-dose fluorescein sodium (20 mg/kg; Alcon Japan Co., Ltd., Tokyo, Japan) is intravenously injected after dural opening at the craniotomy site. We start tumor removal more than 10 minutes after injection of the dye to prevent its leakage with blood from the resected surface. Resection of the tumor is based on identifying the yellow stain (fluorescein sodium), which indicates neoplastic tissue to be resected. We use a conventional microsurgical procedure to resect the tumor with the aid of white-light illumination. The degree of staining from the fluorescein sodium in the lesion is subjectively divided into the three grades (deeply, faintly, and negatively) according to the intensity of the yellow color visible to the naked eye. Our surgical aim is to resect all of the visible yellow color (deeply and faintly stained regions).

**Surgical Procedure and Policy**

After induction of general anesthesia, high-dose fluorescein sodium (20 mg/kg; Alcon Japan Co., Ltd., Tokyo, Japan) is intravenously injected after dural opening at the craniotomy site. We start tumor removal more than 10 minutes after injection of the dye to prevent its leakage with blood from the resected surface. Resection of the tumor is based on identifying the yellow stain (fluorescein sodium), which indicates neoplastic tissue to be resected. We use a conventional microsurgical procedure to resect the tumor with the aid of white-light illumination. The degree of staining from the fluorescein sodium in the lesion is subjectively divided into the three grades (deeply, faintly, and negatively) according to the intensity of the yellow color visible to the naked eye. Our surgical aim is to resect all of the visible yellow color (deeply and faintly stained regions).

**Evaluation of the Extent of Surgery**

The extent of surgery is evaluated by more than three neurosurgeons and one neuroradiologist, excluding the chief surgeon in each case, by viewing postoperative Gd-enhanced T1-weighted MR images obtained 1 month after surgery. Gross-total resection of the tumor is defined as resection with no residual enhancing tumor.

**Histological Examination**

A detailed histological examination of the separately re-
sected surgical specimens at each of the staining grades (deeply, faintly, and negatively) in the same tumor was conducted for four patients by using H & E staining.

Postoperative Adjuvant Therapy

Fifty to 60 Gy of external beam radiotherapy is administered as essential adjuvant therapy following the initial surgery in all patients. External beam radiotherapy was given concurrently with intravenous administration of ACNU (2 mg/kg) in 28 patients. External beam radiotherapy was given concurrently with the intravenous administration of ACNU (2 mg/kg) and interferon-β (120,000 IU/kg) in 26 patients. External beam radiotherapy was combined with the daily intravenous administration of low-dose cisplatin (5 mg/m²) or carboplatin (15 mg/m²) in 51 patients.

Prognostic Factors

To determine the relevant prognostic factors, the following clinical parameters were analyzed: patient age (<40 years compared with ≥40 years), sex, preoperative KPS score (0–60 compared with 70–100), lesioned lobe, tumor size (≤5 cm compared with >5 cm), extent of surgery, fluorescence-guided surgery, and postoperative adjuvant therapy.

Statistical Analysis

The chi-square test was used to evaluate the significance in the difference between GTR rates in patients who underwent fluorescence-guided surgery and those who did not. Survival was analyzed using the method described by Kaplan and Meier, and the significance in differences among the survival curves for each parameter was studied using the log-rank test. A multivariate analysis was performed with the Cox proportional hazard regression model. All statistical analyses were performed with commercially available software (StatView 4.0 for Macintosh computers; Abacus Concepts, Berkeley, CA). Significance was assigned at a probability value of less than 0.05.

Results

Fluorescein Sodium Staining Pattern

The fluorescein sodium staining pattern was evaluated with the naked eye. Immediately after intravenous injection of the dye, the normal cerebral cortex, cortical vessels, dura mater, and tumor were stained yellow. Although the color in the normal cerebral cortex and cortical vessels gradually diminished after approximately 5 minutes, the lesion remained almost homogeneously yellow and the color was intense enough to be readily perceived for resection (Fig. 1). In all cases, the center of the solid lesion was stained a deep yellow and was surrounded by a transition zone that was faintly stained (Fig. 2). This faintly colored area was clearly distinguishable from the negatively stained zone outside the colored lesion, especially in the white matter compared with the cerebral cortex. The cyst fluid of the tumor was intensely colored a fluorescent yellow in all cases. The staining in the lesion lasted for approximately 3 hours.

Histological Findings

The surgical specimen obtained from the deeply stained region included typical GBM histological features, that is, prominent endothelial proliferation, pseudopalisading, and dense tumor cells; the sample from the faintly stained region included less dense tumor cells and scattered endothelial proliferation (Fig. 3A and B). Less dense tumor cells were also consistently revealed in the specimen obtained from the negatively stained area; however, no endothelial proliferation could be seen in this region (Fig. 3C). The same findings were obtained in all four cases examined (Table 3).

Extent of Surgery

Gross-total resection was achieved in 27 patients (84.4%) who underwent fluorescence-guided surgery, which accounted for 100% of those in whom all the visible yellow color (deeply and faintly stained regions) was resected during operation, and in 22 patients (30.1%) who did not receive the fluorescein sodium. There was a significant difference in the GTR rates between these groups (p = 0.0001) (Table 4). Among patients in Stage I, GTR was achieved in 21 (100%) of 21 patients who had been injected with fluorescein sodium, a significantly higher rate than occurred in the 13 (44.8%) of 29 patients who did not receive the dye. Gross-total resection was achieved in five (71.4%) of seven patients who received fluorescein sodium and in eight (50%) of 16 patients who did not among those in Stage II, and in one (25%) of four patients and one (3.6%) of 28 patients, respectively, among those in Stage III. These dif-
ferences in the rates of GTRs between the groups that underwent fluorescence-guided resection and the group that did not were not significant.

Survival Rate and Prognostic Factors

The median survival time and 1-year and 2-year survival rates in patients who underwent fluorescence-guided surgery were 15 months, 66.2%, and 24.1%, respectively, and in those who did not were 13 months, 55.7%, and 15.6%, respectively (Fig. 4). There was no significant difference in survival between these groups (p = 0.0804). In univariate and multivariate analyses for survival, only age (< 40 years), preoperative KPS score (70–100), and extent of sur-

Fig. 2. Case 1. A: Intraoperative photograph depicting a deeply stained region. B: Intraoperative photograph showing both faintly and negatively stained regions and the distinction between the two regions. C: Intraoperative photograph obtained after tumor resection exhibiting the right lateral ventricle and the choroid plexus without any stained region. D: A Gd-enhanced T1-weighted MR image obtained 1 month after surgery, revealing no enhancing tumor.

Fig. 3. Case 1. Photomicrographs of separately resected surgical specimens. A: Prominent endothelial proliferation, pseudopalisading, and dense tumor cells are demonstrated in the deeply stained region. B: Less dense tumor cells and scattered endothelial proliferation appear in the faintly stained region. C: Less dense tumor cells without any endothelial proliferation are revealed in the negatively stained region. H & E, original magnification × 100.
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TABLE 3
Summary of four patients with GBM in whom separately resected surgical specimens were obtained and examined according to the fluorescein sodium staining grade*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age (yrs), Sex</th>
<th>KPS Score</th>
<th>Lobe W/ Lesion</th>
<th>GBM Stage†</th>
<th>Extent of Surgery</th>
<th>Histological Findings</th>
<th>Patient Outcome (mos)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59, M</td>
<td>90</td>
<td>parietal</td>
<td>I</td>
<td>GTR</td>
<td>PP, EP, dense TCs</td>
<td>scattered EP, less-dense TCs</td>
</tr>
<tr>
<td>2</td>
<td>53, M</td>
<td>100</td>
<td>frontal</td>
<td>I</td>
<td>GTR</td>
<td>PP, EP, dense TCs</td>
<td>scattered EP, less-dense TCs</td>
</tr>
<tr>
<td>3</td>
<td>68, F</td>
<td>90</td>
<td>frontal</td>
<td>I</td>
<td>GTR</td>
<td>PP, EP, dense TCs</td>
<td>scattered EP, less-dense TCs</td>
</tr>
<tr>
<td>4</td>
<td>65, M</td>
<td>50</td>
<td>occipital</td>
<td>I</td>
<td>GTR</td>
<td>PP, EP, dense TCs</td>
<td>scattered EP, less-dense TCs</td>
</tr>
</tbody>
</table>

*EP = endothelial proliferation; PP = pseudopalisading; TC = tumor cell.
†For explanation see Table 2 and/or Shinoda, et al.

Discussion

Contrast enhancement of glioma by intravenous administration of fluorescent markers at surgical resection was attempted as early as 1948. These procedures have not been widely or routinely used, however, because of the obscure dyeing pattern during surgery, difficult evaluation of the extent of tumor resection following surgery, and uncertain role of surgery in prolonging survival in patients with glioma. Recently, the role of radical resection in glioma surgery has been stressed as significant for prognosis, and surgical procedures during which fluorescent markers are used for dyeing glioma tissue as an intraoperative tumor navigation technique have been revived. One fluorescent marker is 5-aminolevulinic acid, which is metabolized into strongly fluorescent protoporphyrin IX by a number of malignant tumors in situ through enzymes of the heme-biosynthesis pathway. A high rate of radical resection of GBMs by using 5-aminolevulinic acid visualized under a modified surgical microscope with a special illumination attached filters has been reported on by Stummer, et al. Fluorescein sodium is another fluorescent marker for glioma surgery and is a tracer of BBB disruption and has been clinically used for ophthalmoscopic examinations. Kuroiwa, et al. reported on the functionality of fluorescein sodium in the resection of malignant glioma by using a microscopic system equipped with excitation and barrier filters to observe fluorescence in tissues. Although such fluorescence-guided tumor resection procedures are apparently advantageous in glioma surgery, a surgical microscope must be modified to include a special illumination source and filters are required to visualize tumor-related fluorescence.

The surgical procedure presented in the current report is a simple and readily available fluorescein sodium–guided tumor resection method that does not require any additional equipment other than an ordinary surgical microscope with white-light illumination and uses a higher dose of fluorescein sodium (20 mg/kg) than is generally used (8–10 mg/kg) in ophthalmoscopic examinations.

In this series, the rate of GTR in patients who received fluorescein sodium was significantly higher than that in patients who did not. In particular, the findings that a 100% rate of GTR could be achieved in patients who had undergone the fluorescence-guided surgery and assigned to Stage I of the GBM staging system in which (as a therapeutic policy) the tumor should be resected as radically as possible, were satisfactory and showed the usefulness of this procedure.

Among patients who underwent the fluorescein sodium–guided surgery, GTR was achieved in 100% of patients in whom all the visible yellow color (deeply and faintly stained regions) was resected during operation. This result demonstrates that the visibly yellow lesion observed during surgery completely included the enhancing lesion later demonstrated on MR images. It is generally accepted that the BBB may play an important role in the selectivity of Gad
enhancement of tumor tissue on MR images. The results of this study may support the idea that the lesion stained with fluorescein sodium can also be regarded as the region of BBB disruption and is identical to the Gd-enhanced lesion revealed on MR images. Furthermore, the nonenhancing tumor cannot be stained by fluorescein sodium.

According to the results of histological examinations of the stained lesions, the most significant finding for distinguishing between the visible yellow-colored lesion (deeply and faintly) and the negatively stained region was the existence of endothelial proliferation. These results strongly indicate that endothelial proliferation in GBMs may be involved in BBB disruption. Also based on histological results, leakage of fluorescein sodium into surrounding non-tumorous tissue together with accompanying edema as a possible pitfall of the fluorescence-guided tumor resection procedure was not observed. Note that even in the negatively stained zone surrounding the visibly yellow-colored lesion, a substantial number of tumor cells could be seen. According to these findings, GTR of GBM demonstrated on MR images does not necessarily mean complete resection of all the tumor cells and thus postoperative adjunctive treatment for any residual tumor cells is required.

The patients who underwent GTR showed significantly better survival compared with those who underwent less than GTR. Although survival in the group that received fluorescein sodium was better than that in the group that did not, this difference was not significant. This is not surprising because fluorescein sodium itself is not a therapeutic agent, and GTR was intentionally not achieved in approximately 45% of the patients in Stages II and III, even among those injected with fluorescein sodium, given the surgical risks. On the other hand, GTR was performed in approximately 30% of the patients not injected with the dye. The fact that GTR was one of the significant and independent prognostic factors of GBM regardless of the use of fluorescein sodium during surgery should be stressed.

The yellow color in the skin, mucosa, and urine following the operation was a consistent and fluorescein sodium–related complication in this procedure. The staining completely disappeared 24 hours after the operation, and there was no other critical side effect relevant to the fluorescein sodium. These results indicate that the fluorescence-guided tumor resection is a safe and useful procedure.

**Conclusions**

Although fluorescein sodium–guided tumor resection may be disadvantageous in its subjective color discrimination, the dyed tissue could be seen sufficiently for resection without any special microscopes by using a higher dose of fluorescein sodium than has generally been used in ophthalmoscopic examinations and in previous brain tumor surgeries. This procedure does not require any special surgical microscopic equipment and is simple, safe, useful, readily accomplished, and universally available for resection of GBM. Its efficacy simplifies the surgical procedure of navigating and resecting the stained lesion from the unstained region to achieve GTR of GBM, which can be visualized on MR images.

**References**

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cial study of detection of glioma at surgery using fluorescent imaging by a surgical microscope after fluorescein administration. 


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