Feasibility of simultaneous sodium fluorescein and indocyanine green injection in neurosurgical procedures

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A R T I C L E   I N F O

Article history:
Received 2 March 2016
Received in revised form 23 April 2016
Accepted 3 May 2016
Available online 6 May 2016

A B S T R A C T

Objective: The objective of this study is to assess the feasibility of simultaneous Sodium Fluorescein (SF) and Indocyanine Green (ICG) injection during neurosurgical procedures.

Patients and methods: Three patients harboring a high-grade glioma (HGG) were retrospectively identified in the surgical database of the Neurosurgical Unit 2 at the Foundation IRCCS Istituto Neurologico C. Besta in Milan, by having received intraoperatively both SF for tumor resection and ICG for vasculature angiographic studies in the same surgical procedure. We identified 2 males and 1 female (age range 25–60). Lesions were located in the left temporo-polar area and hippocampus (1 case), right superior frontal gyrus (1 case), left supplementary motor area (1 case). All the three lesions showed Magnetic Resonance Imaging (MRI) characteristics of HGG and, for this reason, in all patients a fluorescein-guided tumor removal was proposed. In the same surgical procedure ICG videoangiography was considered necessary in order to study arterial and venous vasculature, given by the strict relation of the tumor with an unexpected Posterior Communicating Artery (PComA) aneurysm in one case and with cortical drainage veins complexes in the other two cases. In all cases a microscope equipped with both YELLOW560 and Ir800 integrated filters (Pentero 900, Carl Zeiss, Oberkorchen, Germany) was used. Fluorescein was i.v. injected at a dose of 5 mg/kg immediately after patient intubation. ICG was i.v. injected in bolus on demand of the operating surgeon at a dose of 12.5 mg.

Results: No side-effects related to simultaneous injection of SF and ICG were identified. In all three cases, the use of SF allowed to better visualize the tumor areas during surgical removal, thus leading to a radical resection until no macroscopic appearance of residual tumor mass and no fluorescence was visible in the surgical cavity. ICG videoangiography confirmed the patency of branches of internal carotid artery after clipping of an unexpected small PComA aneurysm found intraoperatively during tumor removal in one case, while in patient 2 and 3 it allowed to evaluate patency and study flow pattern in cortical drainage veins that were intimately related to the tumors and the way of the surgical approach. Postoperative MRI showed a Gross Total Resection of the tumors in all cases.

Conclusions: This study showed for the first time the feasibility of intravenous SF injection and ICG videoangiography in the same surgical procedure. The presence of different fluorescence filters on the same surgical microscope allows the surgeon to recognize and safely resect the tumor and simultaneously evaluate local brain vasculization.

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1. Introduction

Surgical removal of high-grade gliomas (HGG) may benefit from the use of fluorescein-guided technique, that allows to better discriminate tumor tissue from normal brain parenchima at the tumor margin, due to its capability to concentrate in areas with blood-brain barrier disruption [1], thus obtaining a high percentage of complete resection [2–5]. Since July 2015, the use of sodium fluorescein (SF) as a tracer for all neurological malignant neoplasm was approved in Italy by the Italian Drug Agency (AIFA) (“Determination n. 905/2015, AIFA”), widening the possibilities of implementation of this fluorofore to all central nervous system (CNS) tumors, enhancing at pre-operative Magnetic Resonance Imaging (MRI).

When operating on CNS tumors, the surgeon may also face the need to evaluate blood vessels in the tumor area and peri-tumor brain parenchima. Indocyanine green (ICG) videoangiography is an established technique to assess brain vascularization intraopera-

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http://dx.doi.org/10.1016/j.clineuro.2016.05.003
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tively by the use of a dedicated filter in the surgical microscope [6–11]. In tumor surgery, it can be used to evaluate tumor vascularization, to confirm arterial patency after tumor resection and to study brain regional perfusion [12–14]. In addition, our group already demonstrated that ICG videoangiography could help in assessing flow dynamic and patency of cortical drainage veins and in evaluating the possibility of venous sacrifice, by the use of a specific clipping test [12,13,15,16]. Furthermore, ICG videoangiography could be also useful for the study of arterial pathologies like intracranial aneurysms, that rarely could coexist with brain tumors [17,18].

Both fluorescence studies require the use of a dedicated microscope, equipped with filters specific for fluorescein and ICG respectively.

In this report we presented three cases in which both SF and ICG were used during the removal of HGGs and we demonstrated for the first time the feasibility of administration in the same patient, during the same surgical procedure, of these two different fluorescences dyes.

2. Patients and methods

2.1. Patients characteristics

Three patients harboring a HGGs were retrospectively identified in the surgical database of the Neurosurgical Unit 2 at the Foundation IRCCS Istituto Neurologo Gino, by having received intraoperatively both SF for tumor resection and ICG for vascular studies in the same surgical procedure. There were 2 males and 1 female (age range 25–60). Patients complained for III nerve palsy, diplopia and short-term memory disturbances (1 case), and seizures (2 cases). Lesions were located in the left temporo-polar area and hippocampus (1 case), right superior frontal gyrus (1 case), left supplementary motor area (1 case) (Fig. 1). All three lesions showed MRI characteristics of HGGs; in particular, a certain degree of contrast enhancement was present in all cases (Fig. 1). For this reason, in all patients a fluorescein-guided tumor removal was proposed, following our specific protocol (see below). In the same surgical procedure, ICG videoangiography was considered necessary in order to: (1) control arterial branches patency after the clipping of an unexpected posterior communicating (PComA) artery aneurysm, found intraoperatively during tumor removal (1 case); (2) evaluate the patency and flow characteristics of cortical drainage veins to the superior sagittal sinuses that were in strict relationship to the tumor and in the way of the surgical approach (2 cases). Histological diagnosis were Anaplastic Oligoastrocytoma in 1 case, Anaplastic Oligodendroglioma in 1 case, and Glioblastoma (GBM) in 1 case (Table 1).

One of the patients included in this analysis was part of the FLUOGLIO phase II trial approved in our Institution (EudraCT No. 2011–002527–18); the other two patients were submitted to fluorescein-guided removal following AIFA determination after July 2015. The surgical database of Neurosurgical Unit 2 at the Foundation IRCCS Istituto Neurologo Gino has been approved by our Ethical Committee.

2.2. Intraoperative fluorescence protocol

2.2.1. Fluorescein administration

Surgical removal of the lesions by fluorescein-guided technique followed the protocol previously published by our group [2–4]. Briefly, 5 mg/kg of SF were injected immediately after patient intubation. Tumor removal was carried out using a microsurgical technique under microscope and fluorescence visualization by YELLOW 560 filter (Pentero microscope, Carl Zeiss, Germany), with the aid of a neuro-navigation system (Stealth S7, Medtronic). Tumor was removed in an inside-out fashion with the aid of ultrasonic aspiration or double aspiration technique, until no fluorescence was left behind, as safely considered feasible by the operating surgeon (FA).

2.2.2. ICG administration

ICG was administrated intravenously as a bolus (12.5 mg in 5 ml of saline), when needed by the surgeon, into a central venous line. The same microscope, equipped also with infra-red sensitive filter and FLOW800 analysis software (Pentero microscope, Carl Zeiss, Germany) was used. Principles of ICG videoangiography using microscope-integrated technology were described in detail elsewhere [6,7,12,13,16]. Briefly, after the injection of ICG the field of interest was illuminated with a near-infrared light and real-time angiographic images were seen on video screen in few seconds, allowing to identify arterial, capillary and venous phases. The fluorescence was cleared within 10–15 min. The resulting video was shown on the microscope screen during surgery and recorded for further visualizations as well. With FLOW 800 software, fluorescence intensities are evaluated in arbitrary intensity units corresponding to the intensity detected by the camera. Based on maximal fluorescence intensities and delay times (time to reach 50% of maximum fluorescence), the software is capable to reconstruct a map in grey-scale (maximal fluorescence intensities) and a colour map (delay time) outlining the blood flow sequence from red to blue. In addition, a pin-point analysis of the course of fluorescence can be performed [16,19].

2.3. Post-operative follow-up

Patients were submitted to early post-operative MRI (within 72 h from surgical procedure) in order to evaluate extent of removal. Clinical general and neurological evaluation were available until hospital discharge, and during the follow-up, including a throughout analysis of possible side effects related to fluorescein administration, as previewed in the FLUOGLIO study for patient 1 and in the AIFA determination for patients 2 and 3.

3. Results

3.1. Fluorescence findings

3.1.1. Fluorescein-guided tumor resection

In all three cases, the use of SF allowed to better visualize the tumor areas during surgical removal. In particular, in patient 1, the tumor (anaplastic oligoastrocytoma) presented as a classical high-grade glioma at pre-operative MRI, with central necrotic core and intense peripheral enhancement on T1 sequences with gadolinium (Fig. 1A). The visualization with YELLOW560 filter allowed to identify the tumor tissue that appeared intensively fluorescent and that was removed in an inside-out fashion until a minimal fluorescent remnant infiltrating the cerebral peduncle was identified and left to avoid damages to the cortico-spinal tract (Fig. 2A,B).

In patient 2, the tumor (anaplastic oligodendroglioma) presented as a huge hyperintense lesion in T2 and FLAIR with areas of contrast enhancement in T1 with gadolinium (Fig. 1C–F). In this case, the tumor presented some superficial fluorescent spots and subcortical areas with intense fluorescent appearance under the YELLOW560 visualization, corresponding to the areas of enhancement at the pre-operative MRI, associated with non-fluorescent tumor areas that corresponded to non-enhancing tumor parts (Fig. 2C,D). Also in this case, the tumor was progressively removed in an inside-out fashion until no macroscopic appearance of residual tumor mass and no fluorescence was visible in the surgical cavity.
**Table 1**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Tumor Localization</th>
<th>Symptoms</th>
<th>Hystological Analysis</th>
<th>Indication for SF injection</th>
<th>Indication for ICG videosangiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Female</td>
<td>62</td>
<td>Left temporo-polar area and hippocampus</td>
<td>III nerve palsy, Diplopia, Short-term memory disturbances</td>
<td>Anaplastic Oligoastrocitoma</td>
<td>Contrast enhancement at preoperative MRI</td>
<td>Control of arterial branches patency after clipping of an unexpected PComA aneurysm that was intraoperatively found</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>24</td>
<td>Right superior frontal gyrus</td>
<td>Seizures</td>
<td>Anaplastic Oligodendroglionioma</td>
<td>Contrast enhancement at preoperative MRI</td>
<td>Patency and flow evaluation of cortical drainage veins to the SSS in strict relation with the tumor</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>57</td>
<td>Left supplementary motor area</td>
<td>Seizures</td>
<td>Glioblastoma</td>
<td>Contrast enhancement at preoperative MRI</td>
<td>Patency and flow evaluation of cortical drainage veins to the SSS in the way of vision of the surgical approach and manipulated during surgical removal</td>
</tr>
</tbody>
</table>

In patient 3, the tumor (GBM) presented as a hyperintense lesion in T2 and FLAIR, with a clear nodule of contrast enhancement in T1 with gadolinium (Fig. 1H–K). Fluorescent visualization with YEL-LOW560 filter allowed to clearly identify the aggressive nodule (Fig. 2E,F). The tumor was progressively removed until no macro-

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*Fig. 1. Preoperative and postoperative MRI of the patients. (A and B): Patient 1. Preoperative axial postcontrast T1 MRI (A) showing a left temporo-polar mass with a necrotic center and peripheral enhancement, involving also the mesencephalic surface. Early (<48 h after surgery) postoperative contrast enhanced axial T1-weighted MRI (B), showing a gross total resection of the lesion. (C–G): Patient 2. Preoperative axial Flair (C), T2-weighted (D), postcontrast T1-weighted (E, F) MRI showing a hyperintense (T2 and FLAIR) – hypointense (T1) lesion in the right superior frontal gyrus, with areas of contrast enhancement (red arrows in E–F). Postoperative axial T1-weighted image with contrast administration (G) showing a complete resection of the lesion. (H–L): Patient 3. Preoperative axial Flair (H), T2-weighted (I), postcontrast T1-weighted (J, K) MRI showing a left hyperintense (T2 and FLAIR) – hypointense (T1) lesion in the left supplementary motor area, with a clear nodule of contrast enhancement (red arrows in J–K). Postoperative axial T1-weighted with contrast image (L) showing a complete resection of the lesion. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)*
scopic appearance of residual tumor mass and no fluorescence was visible in the surgical cavity.

3.1.2. Indocyanine-green videoangiography

In patient 1 ICG videoangiography confirmed the patency of branches of internal carotid artery (PCoM, anterior choroidal artery (ACoA), small perforating branches) after clipping of an unexpected small PCoM aneurysm, found intraoperatively during tumor removal (Fig. 3A,B).

In patient 2 and 3 ICG videoangiography, with FLOW 800 analysis, allowed to evaluate patency and study flow pattern in cortical drainage veins that were intimately related to the tumors and the way of the surgical approach. In particular, in patient 2 the cortical drainage veins remained patent with anterograde flow toward the superior sagittal sinus (SSS) after tumor removal (Fig. 3C,D); in patient 3, instead, the distal segment of the cortical drainage vein thrombosed after tumor removal due to surgical manipulation for an inter-hemispheric approach, but ICG videoangiography demonstrated retrograde flow through a collateral (Fig. 3E,F).

3.2. Clinical and radiological results

Median follow-up was 4 months (range 1–15). No complication related to fluorescein and ICG administration was registered in the three patients included in the study. All patients presented yellow fluorescent discoloration in the urine for 24–48 h. One patient (n.3) presented leg motor weakness (1/5) that improved at 1 month follow-up (4/5). One patient (n.1) died 15 months after a surgical procedure for tumor recurrence. Patients 2 and 3 are alive at the last follow-up, respectively 4 and 1 months after surgical procedure.

All the postoperative MR images showed a Gross Total Resection of the tumors (Fig. 1B,G,I).

4. Discussion

Our study confirmed for the first time the feasibility of simultaneous intravenous SF and ICG injection during neurosurgical procedures. Indeed, the presence of two different fluorescence filters (YELLOW 560 and FLOW 800) in the same surgical microscope (Pentero 900, Carl Zeiss Meditec, Germany) allowed the surgeon to recognize and safely resect tumor tissues, checking and preserving simultaneously local brain vascularization.

In recent times, the implementation of fluorescence in neurosurgery has undergone a great interest by the neurosurgical community, given by the wide range of possible use, from neurovascular to oncological procedures [8,11,20–23].

5-Aminolevulinic acid (5-ALA) and SF are the most used neuro-oncological fluorescent tracers. 5-ALA is a precursor of hemoglobin that causes accumulation of fluorescent porphyrins in tumor cells, including gliomas. Since Slummer et al. presented the results of their phase III trial on 5-ALA guided technique for HGGs resection, demonstrating a positive effect on extent of resection and progression-free survival, it has been established as an important tool for safe and extensive resection in HGG patients [24,25]. SF (the sodium salt of fluorescein), instead, is a fluorochrome that, when injected i.v., has the capability to concentrate in tumor areas of the CNS, due to their induced disruption of the blood-brain barrier [1]. Given its low cost (about five euros per injection), its safety profile, and the preliminary data confirming the efficacy in terms of better tumor visualization by the use of a dedicated filter on the surgical microscope, has recently gained wide diffusion in neurosurgery [2,4,21,22,26–29]. Our group was the first to propose a phase II prospective trial on fluorescein-guided resection of HGGs with a dedicated filter on the surgical microscope (“FLUOGLIO” study) [2] and our report of 2014 on a 20 patients cohort showed a total resection of the contrast-enhancing tissue in 80% of cases [3]. In addition,
preliminary data showed the possibility of using SF with a dedicated filter on the surgical microscope for resection of other types of CNS tumors, including metastasis, lymphomas and hemangioblastomas [22,27–29]. Finally, the use of SF has been also proposed, with a bolus type of injection, in order to visualize intracranial vessels during surgical procedures for arteriovenous malformations resection [30] and aneurysm clipping [31], by the use of an integrated filter on the surgical microscope. Eventually, in July 2015 the use of SF as a tracer for all neurological malignant neoplasm was approved in Italy by the AIFA (“Determina n. 905/2015, AIFA”), widening the possibilities of implementation of this fluorofo to all neurological enhancing tumors at MRI with contrast administration.

In the cases included in this report, the use of SF was considered due to tumor characteristics, with contrast enhancement at the pre-op MRI; in fact, the visualization of tumor under YELLOW 560 filter in the surgical microscope allowed to better visualize the tumor mass and, in case 2 and 3, to identify high-grade spots in the context of the main tumor mass, thus leading to a macroscopic radical removal of the lesion (confirmed in all the cases by a postoperative MRI with intravenous contrast administration).

ICG videoangiography is a simple, low cost and highly effective method that offers real-time information about arterial and venous intracranial vessels, and brain parenchymal perfusion in the area that is exposed during neurosurgical procedures [6,7,9,16,32]. Its use has been implemented in vascular neurosurgery, specifically during aneurysm clipping [11,32,33], arteriovenous malformation removal [34], dural arteriovenous fistulas closure [35], and verification of bypass patency [36]. Our group proposed for the first time the use of ICG videoangiography during tumor removal in order to evaluate its pathological vascularization and check for arterial patency and brain perfusion after resection [12,14]. In addition, we explored the usefulness of ICG videoangiography in studying the characteristics of venous flow and predicting the presence of a safe collateral circulation for veins that are at risk for intended or unintended damage during neuro-oncological procedures. We also designed a specific test (“ICG temporary clipping test”) in which, after a baseline ICG videoangiography, a temporary clip is placed on the vein to be tested and flow direction and dynamic are again analyzed by ICG videoangiography. Flow stagnation is considered to be a sign of lack of collateral circulation whereas flow reversal is interpreted as a sign of the presence of collateral circulation and possibility of venous sacrifice [15,16].

In the first patient of this report, ICG administration was performed because of unexpected intraoperatively finding of a small PCoM aneurysm during tumor resection. Association between intracranial aneurysms and tumors is uncommon (incidence ranged between 0, 3% and 4%) but sometime they could coexist. A combination of GBM and cerebral aneurysms is even rarer [37–40]. The pathogenesis has been debated: a theory proposed that an increase in flow inside pathological low-resistance vessels feeding the tumor could contribute to the development of the aneurysmal [18,37]; another theory suggested that tumor invasion of blood vessels could play a role in the development of vascular malformations as aneurysms [41]. In our case, the aneurysm was found only intraoperatively during tumoral resection in the deep of the surgical cavity. Due to its proximity to the surgical field, rel-
atively easiness of clipping and the thin-wall appearance, it was clipped straightforward. ICG confirmed the complete occlusion of the aneurysmal sac and the preservation of the blood flow in the left PComA, AChO A and other perforating arteries (Fig. 3A,B). In the other two cases ICG videoangiography was used for the study of the superficial venous system, because of tumor location and tumor approach, and relationship with cortical draining veins. Specifically, in case n. 2 the dye was administered during the last steps of the surgery, confirming the patency of two large cortical drainage veins complexed found intimately related to the tumor that could have been unintentionally damaged during the surgery (Fig. 3C,D). Post-operative finding was uneventful. In case n. 3, the videoangiographic study showed the thrombosis of the distal segment of a cortical drainage vein related to surgical manipulation during surgical approach and removal, with a good collateral flow through the proximal segments of the vein complex (Fig. 3E,F). In this case a leg motor weakness related to close proximity to the primary motor cortex developed, that improved at the last follow-up; no venous infarction was found at post-operative MRI.

We recognized the possibility of using SF also for vascular studies in our series of patients. However, in the case n.1, this was not considered because the aneurysm was incidentally found during tumor resection when still some tumor remnant was present. The utilization of a bolus type of SF injection, that causes brain perfusion and intense fluorescent appearance of all normal brain parenchima, lasting minutes after injection [4], could have induced a difficult tumor discrimination, with a significant impact on extent of resection. Furthermore, at least in Italy, SF is not yet approved for usage in vascular neurosurgery. It could be used off-label requiring a specific consent by the patient, or in the context of a specific research program. Thus, even if theoretically in case n. 2 and n.3 SF could have been used with two different indications (tumor resection and vein study after tumor removal), we were not allowed to do so without a specific consent by the patient.

Our study still has certain limitations. First of all, in order to use the two dyes in the same surgical procedure, the microscope (in our case Pentoro 900, Carl Zeiss) needs to be equipped with both surgical filters (specifically YELLOW 560 for fluorescein visualization and FLOW 800 for ICG videoangiography) and this surely increases its cost. We could only hypothesize, due to the lack of specific experience, that an association of 5-ALA and ICG, with a microscope equipped with both filters for these fluorescent tracer (BLU 400 for 5-ALA) could have a similar possible application in neurosurgery, but this is far beyond the scope of this manuscript. In addition, and more importantly, due to the analysis of the procedure in only three patients, larger-scale systematic studies and clinical researches enrolling more patients are required to further assess the value and the benefits given by the use of both these fluorescence technologies during the same surgery. Nevertheless, the aim of the study is not to propose the systematic use of both dyes during resection of brain tumors, but to report the feasibility of using in the same surgical procedures SF assistance and ICG videoangiography, when indicated by the complexity of the specific case.

To our knowledge, this is the first paper showing this possibility.

5. Conclusions

This study showed for the first time the feasibility of intravenous SF injection and ICG videoangiography in the same surgical procedure. The presence of different fluorescence filters on the same surgical microscope allows the surgeon to recognize and safely resect the tumor and simultaneously evaluate local brain vascula-
Technique,


