Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2019 February ; 10862: . doi:10.1117/12.2510905.

On the use of fluorescein-based contrast agents as analogs to MRI-gadolinium agents for imaging brain tumors

Scott C. Davis^a, Margaret R. Folaron^a, Rendall R. Strawbridge^a, Caroline Filan^a, Kimberley S. Samkoe^{a,b}, David W. Roberts^{b,c}

^aThayer School of Engineering, Dartmouth College, 14 Engineering Dr., Hanover, NH USA 03755-8001

^bDepartment of Surgery, Geisel School of Medicine, 1 Rope Ferry Rd., Hanover, NH USA 03755

^cSection of Neurosurgery, Dartmouth-Hitchcock Medical Center, Lebanon, NH USA 03756

Abstract

Magnetic resonance imaging (MRI) of gadolinium (Gd)-based contrast agents plays a central role in managing the treatment of intracranial tumors. These images are involved in diagnosis, surgical planning, surgical navigation, and postoperative assessment of extent of resection. Replicating the information from Gd-MRI in the visual surgical field using fluorescent agents that behave similar to gadolinium in vivo would represent a major advance for surgical intervention of these tumors, and could provide robust compensation information to update pre-operative MRI images during surgery. In this paper, we examine the uptake of a Gd-based contrast agent in orthotopic tumor models and compare this behavior to two fluorescein-based contrast agents; specifically, clinical-grade sodium fluorescein (NaFl) and a 900 Da pegylated form of fluorescein. We show that the pegylated form of fluorescein is a more promising Gd-analog candidate.

Keywords

fluorescence guided surgery; brain tumor; glioma; contrast agents; gadolinium; MRI

1. INTRODUCTION

Surgical resection is a primary treatment modality for intracranial tumors, and there is mounting evidence that maximizing extent of resection during cytoreductive surgery improves outcomes.¹⁻⁴ A central role in the management of these lesions is MRI imaging of gadolinium-based contrast agents, which is involved in diagnosis, surgical planning, intra-surgical guidance and assessment of extent of resection after surgery. Tumor-to-normal contrast, which can be quite high, is generally understood to arise from leakage and retention of the contrast agent in tumor regions which have compromised the blood brain barrier (BBB). During surgery, the MRI images with segmented Gd-defined tumor regions are co-registered to the patient and provide direct guidance and navigation for the surgeon. However, the concordance between these imaged volumes and the tissue is affected by patient positioning and other changes caused by the intervention itself. This brain shift phenomenon is a major challenge for surgical navigation and has been a focus of widespread research efforts to develop compensation techniques. These include intra-surgical ultrasound

or stereovision coupled to deformation models to update the pre-operative MRI, and fluorescence guided surgery to guide tumor resection in the visual field.^{5–9} Combining these approaches may provide a particularly effective solution. Specifically, a fluorescent contrast agent that behaves similar to Gd-MRI could provide an ideal sparse data set for compensatory pre-operative MRI image updating.

Sodium fluorescein (NaFl) is a fluorescent agent approved for clinical use in retinal angiography and has been investigated for decades as a tumor marker for glioma during surgery.^{10–14} However, the observed behavior of this agent in vivo has been mixed, often showing significant uptake in normal tissue. In a recent study, we reported on a comprehensive examination of the kinetics of NaFl in blood, normal brain, and tumor.¹⁵ Specifically, we found that NaFl, which is a relatively small molecule (400 Da) binds only weakly to blood proteins, with a large component remaining in the free state.¹⁵ This unbound NaFl crosses the blood brain barrier while the protein-bound dye remains in the blood. This behavior confounds tumor imaging as the unbound dye in the brain reduces the potential tumor-to-normal contrast. We also found that a modified, pegylated form of fluorescein did not cross the BBB readily.

Herein, we investigate Gd uptake in orthotopic glioma models over time and discuss the potential for NaFl and/or the pegylated form to mimic the kinetics of Gd-based contrast agents, potentially serving as an optical analog to Gd-MRI for surgical guidance.

2. MATERIALS AND METHODS

All animal procedures were approved and conducted in accordance with policies by Dartmouth's IACUC. The orthotopic tumor xenograft model consisted of U251 tumor cells implanted intracranially in athymic nude mice. This is a well-known glioma line that highlights on MRI due to the breakdown of the BBB in the tumor. After implantation of 10^6 cells, the tumors grew for 3–5 weeks, during which the tumors were monitored using MRI. Once tumors were 3–4 mm across, they were put on study.

For MRI imaging, animals were positioned in a customized rodent coil compatible with a Philips Achieva 3T clinical scanner¹⁶. High resolution T1- and T2-weighted images followed by one set of fast T1-weighted images (4 slices) were acquired prior to Gd administration. Dynamic imaging (fast T1W images) began immediately after the administration of gadobutrol in the tail vein and was run for about 70 minutes after administration at a rate of 89 seconds per frame.

Animals in the fluorescence imaging group were administered NaFl or Pegylated fluorescein in the tail vein and then sacrificed at the pre-determined time point after administration, as describe previously¹⁵. Brains were harvested, gross sectioned, and imaged with a Typhoon flatbed scanner. Finally, tissue was processed for H&E analysis to confirm the presence of tumor.

3. RESULTS AND DISCUSSION

Figure 1 shows one T1W slice at several time points after Gd administration from the fast, low resolution dynamic scan sequence. The left column shows the pre-injection image and the remaining images are post-contrast images after subtraction of the pre-contrast images. Notable in almost all images is the strong tumor contrast compared to normal brain (over 10:1 in some cases).

An optical agent that provided similar uptake to gadolinium would be useful for surgical guidance, and could even serve as sparse data for brain shift compensation during surgery. However, our results published previously¹⁵ show that NaFl would be a poor choice given the propensity of the agent to leak into normal brain tissue. Our results showed that tumor to normal contrast was severely limited by this effect. Examples of the NaFl images from representative mice are presented in Fig. 2.

As observed in the images, and previously reported, the presence of NaFl in the normal brain limits the achievable contrast, which is nowhere near as high as observed in the Gd-MRI images (maximum around 2:1). To address this, we proposed the use of fluorescein modified with a short PEG molecule.¹⁵ This molecule was chosen to have a similar size to gadolinium-based chelates used in MRI. Example images of pegylated fluorescein in brain sections are shown in Fig. 3.

These images show much higher tumor-to-background contrast more in line with what was observed in the Gd-MRI images.

4. CONCLUSION

Considering the central role of Gd-MRI in diagnosing, treating, and monitoring intracranial tumors, the introduction of an optical analog to gadolinium-based MRI contrast agents would be a major development for guiding the resection of these lesions. Until recently, an obvious candidate was NaFl, given its long historical use in humans and the ongoing interest in its use for surgical guidance. However, we previously showed that NaFl is a poor candidate for this application due to its behavior in normal brain tissue, which does not correspond to gadobutrol, as shown herein. A pegylated form of fluorescein which does not cross the normal blood brain barrier shows promise as an alternative candidate. Tumor contrast using this form was higher than for NaFl alone, and the observed uptake patterns were closer to those of Gd-MRI. Further studies using co-registered MRI and cryomacrotome fluorescence imaging will be completed to evaluate this and other candidates.

5. ACKNOWLEDGEMENT

This work was sponsored by NIH research grant R01 CA184354.

REFERENCES

- [1]. Lacroix M, Abi-Said D, Fourney DR, Gokaslan ZL, Shi W, DeMonte F, Lang FF, McCutcheon IE, Hassenbusch SJ, Holland E, Hess K, Michael C, Miller D and Sawaya R, "A multivariate

analysis of 416 patients with glioblastoma multiforme: prognosis, extent of resection, and survival,” *J. Neurosurg.* 95(2), 190–198 (2001).

- [2]. Sanai N and Berger MS, “GLIOMA EXTENT OF RESECTION AND ITS IMPACT ON PATIENT OUTCOME,” *Neurosurgery* 62(4), 753–766 (2008). [PubMed: 18496181]
- [3]. Bloch O, Han SJ, Cha S, Sun MZ, Aghi MK, McDermott MW, Berger MS and Parsa AT, “Impact of extent of resection for recurrent glioblastoma on overall survival: clinical article,” *J. Neurosurg.* 117(6), 1032–1038 (2012). [PubMed: 23039151]
- [4]. Ius T, Isola M, Budai R, Pauletto G, Tomasino B, Fadiga L and Skrap M, “Low-grade glioma surgery in eloquent areas: volumetric analysis of extent of resection and its impact on overall survival. A single-institution experience in 190 patients: clinical article,” *J Neurosurg* 117(6), 1039–1052 (2012). [PubMed: 23039150]
- [5]. D’Amico RS, Kennedy BC and Bruce JN, “Neurosurgical oncology: advances in operative technologies and adjuncts,” *J Neurooncol* 119(3), 451–463 (2014). [PubMed: 24969924]
- [6]. Fan X, Roberts DW, Schaewe TJ, Ji S, Holton LH, Simon DA and Paulsen KD, “Intraoperative image updating for brain shift following dural opening,” *Journal of neurosurgery* 126(6), 1924–1933 (2016). [PubMed: 27611206]
- [7]. Stummer W, Pichlmeier U, Meinel T, Wiestler OD, Zanella F, Reulen HJ and LA-Glioma Study Group A., “Fluorescence-guided surgery with 5-aminolevulinic acid for resection of malignant glioma: a randomised controlled multicentre phase III trial,” *Lancet Oncol* 7(5), 392–401 (2006). [PubMed: 16648043]
- [8]. Roberts DW, Miga MI, Hartov A, Eisner S, Lemery JM, Kennedy FE and Paulsen KD, “Intraoperatively updated neuroimaging using brain modeling and sparse data,” *Neurosurgery* 45(5), 1199–1206; discussion 1206–1207 (1999). [PubMed: 10549938]
- [9]. Roberts DW, Valdes PA, Harris BT, Fontaine KM, Hartov A, Fan X, Ji S, Lollis SS, Pogue BW, Leblond F, Tosteson TD, Wilson BC and Paulsen KD, “Coregistered fluorescence-enhanced tumor resection of malignant glioma: relationships between 5-aminolevulinic acid-induced protoporphyrin IX fluorescence, magnetic resonance imaging enhancement, and neuropathological parameters. Clinical article,” *J. Neurosurg.* 114(3), 595–603 (2011). [PubMed: 20380535]
- [10]. Moore G and Peyton W, “The clinical use of sodium fluorescein and radioactive diiodofluorescein in the localization of tumors of the central nervous system,” *Minn Med* 31(10), 1073–1076 (1948). [PubMed: 18888792]
- [11]. Acerbi F, “Fluorescein assistance in neuro-oncological surgery: A trend of the moment or a real technical adjunct?,” *Clin Neurol Neurosurg* 144, 119–120 (2016). [PubMed: 27038874]
- [12]. Acerbi F, Cavallo C, Broggi M, Cordella R, Anghileri E, Eoli M, Schiariti M, Broggi G and Ferroli P, “Fluorescein-guided surgery for malignant gliomas: a review,” *Neurosurg Rev* 37(4), 547–557 (2014). [PubMed: 24756415]
- [13]. Roberts DW and Olson J, “Fluorescein Guidance in Glioblastoma Resection,” *New England Journal of Medicine* 376(18), e36 (2017).
- [14]. Schebesch K-M, Brawanski A, Hohenberger C and Hohne J, “Fluorescein Sodium-Guided Surgery of Malignant Brain Tumors: History, Current Concepts, and Future Project,” *Turkish neurosurgery* 26(2), 185–194 (2016). [PubMed: 26956810]
- [15]. Folaron M, Strawbridge R, Samkoe KS, Filan C, Roberts DW and Davis SC, “Elucidating the kinetics of sodium fluorescein for fluorescence-guided surgery of glioma,” *J. Neurosurg.* 1–11 (2018).
- [16]. Davis SC, Pogue BW, Springett R, Leussler C, Mazurkewitz P, Tuttle SB, Gibbs-Strauss SL, Jiang SS, Dehghani H and Paulsen KD, “Magnetic resonance-coupled fluorescence tomography scanner for molecular imaging of tissue,” *Rev Sci Instrum* 79, 064302 (2008).

Fig. 1.

Pre- and post- gadolinium images in the head of four mice bearing brain tumors over the course of an hour. Tumor regions are outlined in red. Gadolinium images are post contrast minus pre-contrast to emphasize the contrast agent behavior.

Fig. 2.
Sodium fluorescein in tumor-bearing brain sections (ex vivo) 30 and 60 minutes after administration. Tumor-to-normal brain contrast is relatively poor.

Fig. 3.
PEG fluorescein in tumor-bearing brain sections (ex vivo) 30 and 60 minutes after administration.