Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2020 February ; 11219: . doi:10.1117/12.2543921.

Evaluating uptake of multiple fluorescent contrast agents in brain tumors simultaneously using whole animal multi-spectral cryo-imaging

B. K. Byrd, D. J. Wirth, B. Meng, R. S. Strawbridge, S. C. Davis

Thayer School of Engineering at Dartmouth College, 14 Engineering Dr. Hanover, NH, 03755

Abstract

MRI images of gadolinium-based contrast agents (GBCA's) acquired before surgery are often registered to patients and used to guide surgical resection of intracranial tumors. Yet, the accuracy of these MR images in describing the surgical field degrades as surgery progresses; a well-recognized problem which has prompted efforts to develop new techniques that provide updated guidance information on residual tumor location. These efforts span a wide array of technologies, including image updating with deformation models, intraoperative MRI, and fluorescence guided surgery, among others. However, introduction of a straightforward technique that provides surgeons with a current view of GBCA distribution in real time remains an important goal. In this context, development of a fluorescent agent that recapitulates the kinetic behavior of GBCA's could provide familiar information directly in the surgical field in real time. To advance this strategy, we have begun identifying fluorescent contrast agents that show similar kinetic behavior to GBCA's. Using a novel hyperspectral whole body cryo-imaging system, we acquired high-resolution 3-D volumes of the distribution of multiple candidate fluorophores in whole heads bearing orthotopic brain tumors. Preliminary results reveal significant differences in the distribution of candidate optical agents, some of which show strong similarity to the GBCA uptake. Identification and eventual translation of a reliable GBCA-optical analog could improve and simplify surgical resection of brain tumors.

Keywords

whole body molecular imaging; fluorescence guided surgery; cancer imaging; brain tumor

1. INTRODUCTION

The current standard-of-care for resection of intracranial tumors relies heavily on pre-operative imaging^{1,2}. Specifically, MRI imaging of a GBCA is often registered with the patient position in the OR and used to guide surgical resection throughout the procedure, and a lack of GBCA enhancement after surgery defines a complete resection. However, tissue deformation, intracranial pressure changes, and changes induced by the surgery itself reduce the accuracy of these images as the procedure progresses. Efforts to compensate for these changes span a wide range of approaches, including intraoperative MRI, which is often resource-prohibitive, and other image updating strategies. Amongst these efforts, fluorescence guided surgery (FGS) using ALA-PpIX has been widely adopted to provide

real-time tumor guidance during surgery^{3,4}. However, because the mechanisms of contrast arise from different biological processes, the observed PpIX fluorescence distribution does not always agree with the Gd-MRI images.

Herein, we examine an alternative strategy which aims to identify fluorescent imaging agents that closely mimic the pharmacokinetics of extracellular fluid GBCA's. This approach would provide information that is familiar to the surgical community directly in the visual field, providing real-time guidance. In this preliminary study, we examined the distribution of three potential fluorescent imaging agents simultaneously at high resolution in whole animals. Specifically, we administered three spectrally-distinct imaging agents and a GBCA simultaneously to animals with xenograft brain tumors, and used a novel multi-spectral whole-body imaging cryo-macrotome to produce high resolution volumetric images of all agents⁵. Comparing the uptake kinetics of each agent, we discuss the current status and potential to translate this optical-GBCA analog strategy.

2. METHODS AND MATERIALS

2.1. Animal preparation and experimental design

All animal procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee at Dartmouth College. Figure 1 provides the general experimental flowchart. Glioma U251 cells were intracranially implanted into mouse models and monitored via MR imaging until the glioma tumor size reached greater than 5 mm in diameter. Once tumors reached this size, animals were positioned in an MRI rodent RF coil in a clinical 3.0T Philips scanner. Pre-contrast administration MRI images were acquired (T1-weighted) and then a solution containing both GBCA and three fluorescent agents was administered in the tail vein. This solution consisted of 0.5 mM/kg Gadavist (Bayer HealthCare Pharmaceutical, Whippany, NJ, USA), a 5kDa form of PEGylated Fluorescein (PEG-FITC, 12 mg/kg human-equivalent dose) (Creative PEGWorks, Chapel Hill, NC), IRDye® 680RD conjugated to Affibody negative control (1 μ M) (LI-COR Biosciences, NE, USA; Affibody Medical, Solna, Sweden), and clinical-grade Indocyanine Green (1mg/kg human-equivalent dose) (Thermos Fisher Scientific, MA, USA). After administration, MRI images were acquired at a designated time (between 5 and 60 minutes) and the animal sacrificed, embedded in optical cutting temperature (OCT) material and frozen to -20°C for cryo-imaging.

2.2 Imaging cryo-macrotome

The whole body imaging cryo-macrotome consists of a Leica CM3600 cryo-macrotome modified with a series of LED and laser excitation sources, a motorized filter wheel, a liquid crystal tunable filter, and two scientific CMOS cameras for white light and multi-spectral fluorescence imaging. In this study, we used a 470 nm LED to image PEG-FITC, a 635 nm laser to image IRDye-680-Affibody NC, and a 760 nm laser to image ICG uptake. Frozen specimens positioned in the macrotome are sectioned automatically and multi-spectral data acquired for each channel after each slice. Images of each slice are then processed via dark image subtraction and flat field correction and reconstructed into 3-D

volumes for each channel using both NIRFAST Slicer (Kitware, Inc, Clifton Park, NY) and ImageJ (National Institute of Health, Bethesda, MD).

3. RESULTS

Two dimensional images from the 3-D stack are shown in Fig. 2 (a–d) for white light and all three fluorescent channels in one animal 15 minutes after administration. These images show dramatic differences in the distribution of fluorophores in the brain, head and tumor even at this early time point.

Figure 3(a–c) shows whole body slices of each fluorescence channel overlaid on the corresponding white light images. Again, the clearance and biodistribution of these untargeted agents differ significantly, with ICG accumulating in the liver, and PEG-FITC and IRDye@680-Affibody both accumulating in the kidneys, though with different distribution patterns. T1-weighted Gd-MRI images show the GBCA distribution, acquired immediately before euthanasia, at the same time point in the head. Preliminary evaluation of agent distribution in tumor suggests the PEG-FITC molecule most closely resembles GBCA uptake at this time point. ICG had lowest tumor-to-normal contrast of the three agents, again, at this early time point.

4. DISCUSSION AND CONCLUSION

These preliminary results validate the evaluation strategy for identifying and developing optical-GBCA analogs with high fidelity. Specifically, the use multi-spectral cryo-imaging enables the assessment of the distribution of multiple fluorescent agents in whole animals simultaneously at 100-um-level resolution in all three dimensions. As shown herein, agents have dramatically different uptake patterns in vivo, even at very short administration times, and at least one agent showed strong correspondence with Gd-MRI.

The information contained in Gd-MRI is central to diagnosis and surgical guidance for treatment of gliomas. Identification of a suitable optical-GBCA analog could replicate this information directly in the surgical field, robust to tissue deformation. Importantly, the administration times used for Gd-MRI are quite short. Thus, an optical analog would permit the use of similarly-short administration times, a major practical advantage over current FGS agents that require 3–24 hr. incubation times. Further evaluation and development are ongoing.

ACKNOWLEDGEMENTS

This work was funded by the National Institute of Health grants R01CA184354 (SC Davis).

REFERENCES

- [1]. 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,” *Journal of Neurosurgery* 117(6), 1032–1038 (2012). [PubMed: 23039151]
- [2]. Sanai N, Polley M-Y, McDermott MW, Parsa AT and Berger MS, “An extent of resection threshold for newly diagnosed glioblastomas: Clinical article,” *Journal of Neurosurgery* 115(1), 3–8 (2011). [PubMed: 21417701]

- [3]. Roberts DW, Hartov A, Kennedy FE, Miga MI and Paulsen KD, "Intraoperative brain shift and deformation: a quantitative analysis of cortical displacement in 28 cases," *Neurosurgery* 43(4), 749–758; discussion 758–760 (1998). [PubMed: 9766300]
- [4]. Valdés PA, Fan X, Ji S, Harris BT, Paulsen KD and Roberts DW, "Estimation of Brain Deformation for Volumetric Image Updating in Protoporphyrin IX Fluorescence-Guided Resection," *SFN* 88(1), 1–10 (2010).
- [5]. Meng B, Byrd BK, Wirth DJ, Strawbridge RR and Davis SC, "Developing a novel hyperspectral imaging cryomacrotome for whole body fluorescence imaging," *Visualizing and Quantifying Drug Distribution in Tissue IV 11219, 112190M*, International Society for Optics and Photonics (2020).

Figure 1.

Flowchart showing the procedure to assess GBCA and fluorescent agent uptake kinetics in glioma models.

Figure 2.

(a) Representative white light image alongside channel-specific fluorescence images for PEG-FITC (b), IRDye680-Affibody NC (c), and ICG (d).

Figure 3. Sagittal view of fluorescence images overlaid on corresponding white light images in each of the fluorescence channels: (a) PEG-FITC, (b) IRDye680-Affibody NC and (c) ICG channels. (d) Post-contrast T1-weighted MR image of the same mouse prior to cryo-imaging.