Published in final edited form as:

Proc SPIE Int Soc Opt Eng. 2019 February ; 10874: . doi:10.1117/12.2510515.

Noninvasive imaging of dual-agent uptake in glioma and normal tissue using MRI-coupled fluorescence tomography

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Abstract

As the role of immuno-oncological therapeutics expands, the capacity to noninvasively quantify molecular targets and drug-target engagement is increasingly critical to drug development efforts and treatment monitoring. Previously, we showed that MRI-coupled dual-agent fluorescence tomography (FMT) is capable of estimating the concentration of epidermal growth factor receptor (EGFR) in orthotopic glioma models noninvasively. This approach uses the dynamic information of two fluorescent agents (a targeted agent and untargeted isotype) to estimate tumor receptor concentration in vivo. This approach generally relies on the two tracers having similar kinetics in normal tissues, which may not always be the case. Herein, we describe an additional channel added to the MRI-FMT system which measures the uptake of both agents in the normal muscle, data which can be used to compensate for differing kinetic behavior.

Keywords

Dual-agent imaging; fluorescence tomography; MRI-coupled optical imaging; receptor-targeted therapy; uptake kinetic; orthotopic glioma small animal model

1. INTRODUCTION

The ability to quantify cell surface receptors in tumors in vivo will be an important development for preclinical drug development and clinical monitoring of drug-target engagement. Over the past decade, we have advanced a paired-agent imaging approach which uses an untargeted contrast agent administered simultaneously with the targeted agent to compensate for nonspecific uptake in the tumor. To do this, the dual-agent approach images the dynamics of two spectrally distinct fluorescent imaging agents and uses those curves in a paired agent kinetic model [1, 2]. This allows estimation of a parameter known as binding potential, which is proportional to the concentration of receptors available for binding.

We have previously demonstrated this technique in orthotopic glioma models using MRI-coupled fluorescence molecular tomography, a noninvasive, volumetric imaging modality. These studies demonstrated the feasibility of this imaging modality to estimate receptor

density in vivo, but assumed that the kinetics of the two imaging agents in normal tissue were identical, which may not always be the case. However, recent developments in planar imaging studies have shown that the kinetics in normal muscle tissue can be used to accommodate any differences in the paired agent behavior through a deconvolution schema. Herein, we describe an update to the MRI-FMT instrumentation that allows normal muscle data to be acquired with the tomographic data of the head. This development will enable imaging agent differences in arterial input function and other factors like binding independent delivery and retention to be corrected, allowing more accurate assessment of the binding specific kinetic [3].

2. METHODS

2.1 Cell Culture

The glioma cell line chosen for the experiment was: U251 glioma cells (moderated EGFR expressing) [4]. Cells were maintained in DHEM culture media supplemented with 5% fetal bovine serum and 1% antibiotic at 5% CO₂, 37 °C.

2.2 Mouse handling

Animal studies were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) at Dartmouth College. A nude mouse was orthotopically implanted with 1×10^6 tumor cells, as previously described [5]. Approximately 3–5 weeks post tumor implantation, mice were imaged with Gd-MRI (Philips Achieva 3.0T) to confirm presence of orthotopic tumor. A mouse bearing glioma, visible in contrast-MRI and at least 2 mm across, was selected for the study. Twenty-four hours prior to MRI-FMT imaging 1mg of Cetuximab (2mg/mL concentration) was administered to the mouse.

2.3 Imaging agents

The imaging agents used consisted of an anti-EGFR Affibody conjugated to Licor IRDye800CW and Affibody negative control conjugated to Licor IRDye680RD as the targeted and untargeted agents, respectively. For MRI-FMT studies, the dual pair imaging agents (concentration of .2nmol per agent) and the MRI contrast agent Gadovist (.5mmol/kg) (Bayer, Leverkusen, Germany) was mixed in an injection solution of 200µl.

2.4 Dual-agent MRI-FMT acquisition

The MRI-coupled FMT system set-up is explained extensively in previous publications [1,6]. The detection system consists of Acton Research Insight spectrometers with Princeton Instruments PIXIS cameras. Full tomography is taken with 8 optical fibers that circumscribes the mouse head. For this study, another set of two optical fibers were positioned to pinch the leg muscle tissue to record normal tissue kinetics through the leg. Both targeted and untargeted imaging agents were acquired continuously for around 60 minutes after administration, at a rate of approximately 1 minutes and 40 seconds per frames for a total of 35 frames. At each frame, a full set of tomography data around the head as well as transmission measurements through the leg, for both dyes, were recorded. Data pre-processing is accomplished by performing a linear least squares spectral fit to extract the

dye-specific fluorescence from background fluorescence. This fitting is also applied to the projection data from the leg.

2.5 MRI-FMT data processing

The FMT reconstruction process using MRI-spatial priors has been described extensively elsewhere [1, 6]. Briefly, the projection data is used with a diffusion-based reconstruction algorithm (NIRFAST, [7, 8]) on a segmented MRI template. The spectral discrimination between the dyes provides full tomographic data for each agent at each frame. Thus, the reconstruction algorithm provides a volumetric reconstruction of each agent at each frame. Thus, volumetric images of each dye are produced for the 35 frames.

3. RESULTS

An illustration of the FMT system on the head is shown in Figure 1. Optical fibers circumscribe the tumor site at different projections for tomographic reconstruction. Each optical fiber can be excitation source emitting 730 nm or 658 nm laser. The FMT system utilizes the dual imaging agents by extracting their distinctive spectral information through spectral fitting (Figure 1a). Combining the optical data and MRI data, the fluorescence activities were reconstructed to reveal targeted and untargeted imaging agents' behaviors in different regions of the brain. The time series at a few sample time points for untargeted and targeted imaging agents overlaid with MRI is shown in Figure 1b. Untargeted imaging agent cleared out faster than the targeted imaging agent in the brain and tumor region. Tumor region shows the highest fluorescence signal contrast to the brain at early time points for untargeted imaging agent. For the targeted imaging agent, the fluorescence signal is consistent the whole-time series.

The dual-agent uptake kinetics are plotted in Figure 2. Targeted imaging agent kinetic curve is relatively stable with maximum and minimum fluorescence activities of 2.94×10^{-5} and 2.85×10^{-5} (figure 2a). The untargeted imaging agent shows fast clear out in tumor with maximum fluorescence activity (3.25×10^{-5}) at first time point, and minimum fluorescence activity (8.62×10^{-6}) at last time point (Figure 2b). At initial time points, untargeted imaging agent has higher signal intensities than the targeted imaging agent, but after 7 minutes, the targeted imaging agent signal exceeds the untargeted imaging agent signal. Both kinetic curves are normalized to first time point and plotted together (Figure 2c). The differences in signal intensity between the two imaging agents increases with time.

The normal tissue fluorescence activities are collected simultaneously with tomography, and the fluorescence activities for both imaging agents are included in Figure 3. Targeted imaging agent shows slight decrease in signal intensity with maximum and minimum fluorescence activities of 1.07×10^7 and 9.95×10^6 (Figure 3a). Untargeted imaging agent shows significant decrease in signal intensity with maximum fluorescence activity at early time points (1.36×10^6) and minimal fluorescence activity at last time point (9.95×10^5) (Figure 3b). Similar to the uptake curves in tumor, when plotting the normalized fluorescence activities of both dye, targeted agent shows stable intensity in normal tissue, and untargeted agent shows drastic decrease in signal intensities.

4. CONCLUSION

The addition of normal tissue uptake for both dyes will allow deconvolution of the tumor curves with the normal tissue curves. This step, which has been previously reported in planar imaging implementations of paired agent imaging, but not in paired agent FMT imaging, improves the accuracy of binding potential metrics by compensating for differences in kinetics of the two dyes. Work to leverage these data in a deconvolution schema are ongoing.

ACKNOWLEDGMENTS

This study is funded by National Institutes of Health and National Cancer Institute R01 grant (#CA184354) awarded to S.C. Davis.

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Figure 1:

MRI coupled FMT system data acquisition. (a) 3D rendering of the MRI with fibers location marked as blue dots. Two laser sources were used: 730 nm laser for targeted fluorescence agent excitation and 658 nm laser for untargeted fluorescence agent excitation. Two fibers are used to image normal tissue where the fiber probes are pinching on the leg muscle tissue. One fiber emits the excitation lasers, and the other fiber is connected to camera. Spectra of the emission light are collected, and spectral fitting is performed to quantify fluorescence signal intensity for each fluorescence agent. (b) Head tomography for targeted and untargeted imaging agent fluorescence activities reconstructed and overlaid with MRI at different time points.

Figure 2:
Dual-agent uptake kinetics in tumor. (a) Targeted imaging agent reconstructed fluorescence activity. (b) Untargeted imaging agent reconstructed fluorescence activity. (c) Normalized activities.

Figure 3:
Dual-agent uptake kinetics in normal tissue. (a) Targeted imaging agent fluorescence activity. (b) Untargeted imaging agent fluorescence activity. (c) Normalized activities.