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Applying dynamic contrast enhanced MSOT imaging to intratumoral pharmacokinetic modeling

Ted G. Xiao[a], Jared A. Weis[a], F. Scott Gayzik[a], Alexandra Thomas[b], Akiko Chiba[c], Metin N. Gurcan[b], Umit Topaloglu[d], Abhilash Samykutty[d], Lacey R. McNally[a,d,*]

[a] Department of Biomedical Engineering, Wake Forest School of Medicine, Winston-Salem, NC 27101, United States
[b] Department of Internal Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27013, United States
[c] Department of Surgery, Wake Forest School of Medicine, Winston-Salem, NC 27013, United States
[d] Department of Cancer Biology, Wake Forest School of Medicine, Winston-Salem, NC 27013, United States

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ABSTRACT

Examining the dynamics of an agent in the tumor microenvironment can offer critical insights to the influx rate and accumulation of the agent. Intratumoral kinetic characterization in the *in vivo* setting can further elucidate distribution patterns and tumor microenvironment.

Dynamic contrast-enhanced Multispectral Optoacoustic Tomographic imaging (DCE-MSOT) acquires serial MSOT images with the administration of an exogenous contrast agent over time. We tracked the dynamics of a tumor-targeted contrast agent, HypoxiSense 680 (HS680), in breast xenograft mouse models using MSOT. Arterial input function (AIF) approach with MSOT imaging allowed for tracking HS680 dynamics within the mouse. The optoacoustic signal for HS680 was quantified using the ROI function in the ViewMSOT software. A two-compartment pharmacokinetics (PK) model constructed in MATLAB to fit rate parameters. The contrast influx (k_{in}) and outflux (k_{out}) rate constants predicted are $k_{in} = 1.96 \times 10^{-2}\,s^{-1}$ and $k_{out} = 9.5 \times 10^{-3}\,s^{-1}$ ($R = 0.9945$).

1. Introduction

Multispectral optoacoustic tomography (MSOT) is a novel *in vivo* imaging modality with high spatial and temporal resolution at clinically significant detection depths [1–3]. Near-infrared (NIR) light pulses are absorbed by chromophores in the tissue, leading to heat production and thermoelastic expansion. This results in acoustic signal production which can be detected by an ultrasound transducer. MSOT imaging takes advantage of the optoacoustic (OA) effect with ultrasound detection of optical excitation [4]. NIR light with wavelength between 600–900 nm do not attenuate in most tissues, resulting in high resolution images at improved depth than optical imaging [1]. The use of OA overcomes the main limitations of optical and ultrasound imaging including limited tissue penetration (< 8 mm), and lack of potential for molecular information using a wide array of contrast agents [5,6].

Because each chromophore absorbs light and emits a distinct optoacoustic signal, it is possible to detect multiple chromophores simultaneously in high-resolution images as well as to obtain biological information, such as oxygenation state and tissue inflammation [2,7–10]. This ability to simultaneously track multiple chromophores based on absorption spectra allows for one multispectral scanning session to yield multiple images depicting the biodistribution of each chromophore [11–13]. Thus, endogenous and exogenous agents can be used in combination to facilitate the identification of tumors. One hallmark of tumors is a region of tissue hypoxia from abnormal microvessel formation and decreased diffusion, leading to lack of vascular flow [14]. HypoxiSense 680 (HS680) targets and binds to carbonic anhydrase IX, a marker for tumor hypoxia [15]. HS680 absorbs at 670 nm and emits at 685 nm (Fig. 1), which can be tracked using MSOT imaging along with oxygenated hemoglobin (HbO_2).

In addition to detecting distinct spectral signatures, optoacoustic imaging is an intrinsically fast imaging technique [16]. High temporal resolution allows the advantage of visualizing dynamic, biological events, which is difficult in ultrasound imaging due to motion artifacts [17–20]. One application is a pharmacokinetics (PK) study. Pharmacokinetic modeling maps out the kinetics of an administered drug or contrast agent in the plasma of an organism, thus playing a predictable role in the dose-response relationship in toxicity assessment [21–23].

* Corresponding author at: Department of Cancer Biology, Department of Bioengineering, Wake Forest School of Medicine, 1 Medical Center Blvd, Winston-Salem, NC 27157, United States.
E-mail address: lacey_mcnally@hotmail.com (L.R. McNally).

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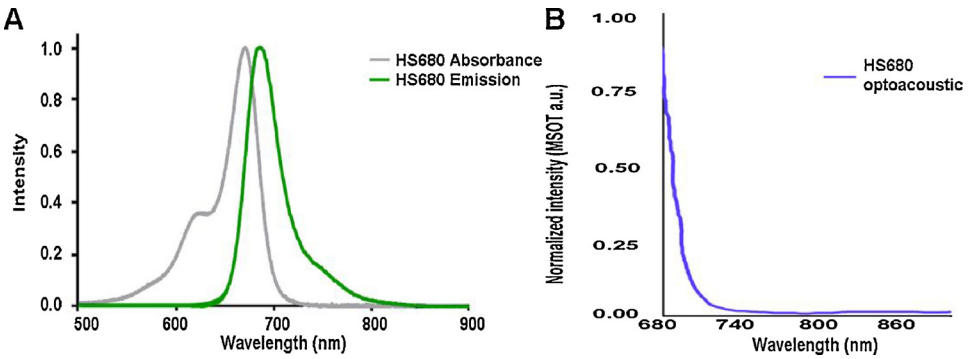

Fig. 1. Spectral signatures for HS680. The absorbance, emission, and optoacoustic spectra were evaluated for HS680. (A) The absorbance and fluorescent emission of HS680 was determined using a spectrofluorometer. (B) The optoacoustic spectrum was determined by inserting HS680 into a tissue mimicking phantom and measured using MSOT. The optoacoustic spectrum was utilized to identify HS680 in vivo as detected using MSOT.

Here we used a 2-compartmental PK model to simplify the body of an organism into one or two compartments, a commonly used approach [24,25]. *in vivo* data are collected and fitted against a PK model with estimated rate parameters.

Combining the functionality of contrast agents with a fast tracking imaging modality yields a powerful tool that can elucidate contrast kinetics and agent biodistribution *in vivo* [18]. DCE-MSOT has not been used previously to investigate PK with a contrast agent targeting tumor hypoxia [18,26]. This study utilizes HS680-MSOT contrast-imaging to study intratumoral pharmacokinetics in the *in vivo* setting.

2. Methods

2.1. Materials

The powder form of HS680 (Perkin-Elmer Inc., Waltham, MA, USA) was diluted to a concentration of 6.1 mM in saline solution. The absorbance and fluorescent emission of HS680 were measured using a FP-8300 spectrofluorometer (Jasco, Oklahoma City, OK, USA) and the OA spectra was measured using an MSOT inVision 256-TF (iThera Medical GmbH, Munich, Germany) within a tissue mimicking phantom as in previous studies (Fig. 1) [8,27]. A tail vein catheter was set up to inject each mouse with 100 µL of HS680 and a concentration of 4.5 µmol/kg mouse and followed by a catheter flush with 100 µL of saline solution. Each injection took ~30 s.

2.2. Mouse breast tumor model

Three female, athymic mice, weighing 20 g each, were injected with 1×10^6 MDA-MB-231 cells in the mammary fat pad. After injection, mice were housed under pathogen-free conditions following the guidelines of the American Association for Laboratory Animal Care. When tumors were 7 mm in diameter (45 days post injection), PK and MSOT imaging studies were performed in accordance with the current standards of the University of Louisville Animal Care Facility, the Institutional Animal Care and Use Committee of the University of Louisville, the National Institutes of Health, the U.S. Department of Agriculture, and the U.S. Department of Health and Human Services.

2.3. Multispectral optoacoustic tomography (MSOT) imaging

A commercially available MSOT inVision 256-TF (iThera Medical GmbH, Munich, Germany) was used for optoacoustic measurements. The MSOT emitted near-infrared laser pulses at 10 Hz and wavelengths between 680 to 900 nm A 256 element, toroidal, ring-shaped transducer received optoacoustic signals. This 270° transducer setup allowed for cross-sectional tomographic imaging with an in-plane resolution of 75 µm which has been further confirmed in our previous manuscript [28], and a center frequency of 5 MHz. This arrangement provides clear visualization of the spatial distribution of injected agents in the animal, avoiding the "masking" of deeper pixels by contrast uptake in a shallow region. In addition, implantation of the orthotopic breast tumor in the mammary fat pad avoided high signal noise level from internal organs.

A mouse was anesthetized with 1.5% isoflurane and 0.8 l medical air with 0.1 l O_2 gas in preparation for imaging. A 27 gauge catheter was inserted in preparation for tail vein injection. To limit physiological changes in the animal and manual introduction of vessel volume fluctuation, we ensured that mouse physiology was stable for 10 min while the mouse was located within the MSOT unit before data collection and performed bolus injection over 30 s followed by a slow saline flush [29]. MSOT signal data were collected 5 min prior to injection of HS680, providing a baseline reading in the tumor region that represents the hemoglobin level. Multispectral images were acquired with three contiguous image slices centered on the tumor for 20 min following injection of HS680 using three wavelengths 680, 760, and 900 nm and 5 pulses per wavelength.

Advanced Molecular Imager (AMI-1000X) was used to detect the biodistribution of HS680 in the mouse model 24 h after injection.

2.4. Image reconstruction and spectral unmixing

A standard back projection algorithm from the ViewMSOT 3.6 software suite (iThera Medical) was used to reconstruct the images [30]. The linear spectral unmixing method was used to detect and separate unique signals for contrast agents (i.e. hemoglobin and HS680) [31,32] by comparing the optoacoustic signals measured at each pixel with the known absorption spectra of the target contrast agent(s), allowing for separation and visualization of the distribution of each agent within the animal. In the current study, this method was utilized to distinguish between signal differences in HS680 and hemoglobin in the tumor and blood vessels.

2.5. Pharmacokinetic modeling

The PK model for this study is a 2-compartmental PK algorithm custom-designed using MATLAB. The two compartments represent the plasma and the breast tumor microenvironment (Fig. 2). The mathematical approach is similar to the 2-compartmental models previously utilized in previous DCE-MRI and DCE-PET studies [33,34]. A differential equation is used to describe the kinetics of HS680 within the tumor microenvironment (Eq. (1)). At a given time point, $C_{tumor}(t)$ is the concentration (nM) of HS680 in the tumor microenvironment; $C_{plasma}(t)$ is the concentration of contrast in the plasma. The rate constant k_{in} (s^{-1}) describes the rate at which HS680 leaves the plasma and is taken up into the tumor space and k_{out} describes the rate at which the agent returns from tumor space into the plasma. The differential equation assumes a known working concentration, which is 0.91 mM of HS680.

$$\frac{dC_{tumor}(t)}{dt} = k_{in} C_{plasma}(t) - k_{out} C_{tumor}(t) \quad (1)$$

This *in vivo* experiment measures optoacoustic signal of HS680.