

Early Detection of Tumor Vascular Response to Anti-Angiogenic Drugs with Optical Tomography

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Abstract: Using optical tomography we have imaged early vascular responses to anti-angiogenic treatments in a small animal tumor model. Optical images acquired from 1 to 7 days after drug administration show measurable changes in hemoglobin concentration.

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1. Introduction

Over the past thirty years there has been considerable research devoted to anti-angiogenic agents and their use in cancer therapy. Many of these agents target growth factors that promote angiogenesis, and in particular vascular endothelial growth factor (VEGF). In 2004 the FDA approved the first anti-vascular drug (bevacizumab) for the use in colon cancer after researchers showed that the use of bevacizumab substantially increased the time to progression [1,2]. Although the clinical results from these agents have been promising, there appears to be a large variability in the effectiveness for various types of cancer, and even for various trials on the same tumor type [1,3]. Given this variability, there is a need for a non-invasive method to predict the outcome of the therapy as early as possible.

It has been shown that in some cases anti-angiogenic drugs can induce the regression of the existing vasculature as early as 24 hours after administration [4-7]. There is some indication that these early responses may correlate with outcomes of long-term therapy. We hypothesize that optical tomography (OT) may offer a non-invasive means to assess early tumor vascular response to anti-angiogenic drugs. OT has been shown to be sensitive to oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb) and preliminary studies on a limited number of mice that were followed for 24 hours after drug injection showed that these early changes may be detectable [8,9]. Indeed changes in transmitted light intensity were observed as early as 30 minutes after injection of the drug. In the study at hand, we employ a Ewing sarcoma tumor model, which is known to respond well to treatment with bevacizumab. The goal of the study was to demonstrate that OT can detect changes in the vasculature within the first week of treatment. Using 10 animals, we performed OT measurements at 1, 3, 5, and 7 days after an initial injection with bevacizumab. We observed a statistically significant drop in total hemoglobin within 72 hours of treatment.

2. Methods

2.1 Instrument and Imaging Probe

The experiments in this study were performed using a digital optical tomography system previously designed and developed in our laboratory [10]. The system utilizes 16 source fibers and 32 detector fibers that are arranged symmetrically in two rings surrounding the imaging cylinder. Each source fiber is sequentially illuminated with two wavelengths of $\lambda_1 = 760\text{nm}$ and $\lambda_2 = 830\text{nm}$, while the 32 detector fibers simultaneously measure the scattered and reflected light through the cylinder. The source and detector fibers are brought into contact with an optical imaging probe consisting of a hollow Delrin center (height = 10cm, diameter = 4.1cm and 3.2cm, wall thickness = 0.15cm) and two fiber-holding rings to bring the fibers in contact with the surface of the cylinder. Each ring holds 24 fibers spaced 15 degrees apart allowing 8 source and 16 detector fibers per ring to be arranged in an alternating pattern of source-detector-detector-source. The two rings are separated by 1.25cm. The cylinder was filled with 1% Intralipid matching fluid to reduce edge effects in the reconstruction.

The key features that make this system ideal for this study are the large dynamic range (~190dB) and imaging frame rates (>5 frames/second) that enable fast and sensitive measurements of the absorption and scattering after the bevacizumab injection [10]. In these experiments one source fiber and one detector fiber were removed from the cylinder and used as a reference signal to account for any variations in the source power over time.

2.2 Experimental Procedure

10^6 cultured human Ewing sarcoma cells engineered to express luciferase (SK-NEP1-*luc*) were implanted intrarenally in NCR nude mice and allowed to grow until the tumor size reached approximately 1g as assessed by biweekly bioluminescence measurements with a Xenogen IVIS apparatus. The treatment schedule consisted of 20 mg/kg bevacizumab injected intravenously every 3 days. Full optical tomographic data sets were collected every 0.2 seconds, starting 5 minutes prior to injection until 50 minutes after drug injection on the first day. During the first drug injection an IV catheter was inserted into the tail vein of the mouse that allowed us to administer the treatment during the optical scan without changing the position of the animal. Each mouse was positioned in the cylinder so that the tumor was located in between the two rings. In addition, 1000 frames of full-tomographic data sets (approximately three minutes of imaging) were acquired at 1, 3, 5, and 7 days after the initial injection. A 300 point subsection of this data with minimal motion was taken and averaged to remove any respiratory and other noise. After the data was acquired with the mouse in the cylinder a reference set of data was acquired for a homogeneous medium of 1% Intralipid. During all imaging procedures the animals were anesthetized with isoflurane gas.

2.3 Reconstruction Algorithm

For reconstructing 3-dimensional spatial distributions of deoxy- and oxy-hemoglobin concentrations ([Hb] and [HbO₂]) we employed a PDE-constrained SQP algorithm that uses the equation of radiative transfer (ERT) as a forward model of light propagation in tissue. We incorporated into a previously presented algorithm [11] a multispectral method that allowed for direct reconstruction of [Hb] and [HbO₂], without first calculating the absorption coefficients, μ_a , at λ_1 and λ_2 . We found that the multispectral method provides more accurate results than the traditional two-step method. From the three-dimensional reconstruction the slice with the maximum chromophore values was extracted for visualization.

3. Results

3.1 Imaging Results

Overall we imaged eight tumor-bearing mice and two healthy control mice for this study. For each mouse images of the oxy, deoxy, and total hemoglobin were reconstructed for inspection. In all of the tumor-bearing mice imaged in this experiment there was a decrease in total hemoglobin seen over the first three days. Seven of the mice showed a strong decrease in the total hemoglobin concentration by day 3. This decrease was followed by an increase in the total hemoglobin at day 5 in five of the seven mice and at day 7 in the other two mice. In some mice, such as the two shown in Figure 1, the rapid return of the total hemoglobin at day 5 could be a result of a known refractory effect termed vascular normalization that occurs as remodeling of larger vessels is induced by loss of VEGF signaling [12]. These findings were confirmed through immunostaining for the well-established endothelial marker PECAM-1, and vascular mural cell marker alpha-Smooth Muscle Actin.

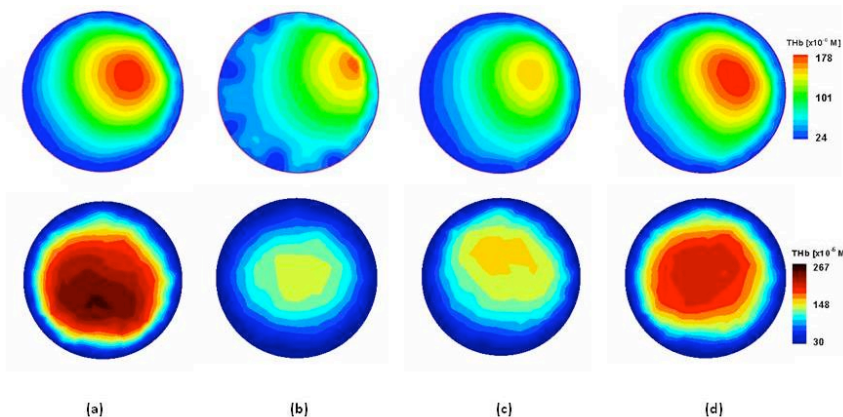


Figure 1. Total Hemoglobin concentration at four time points (a) prior to injection (b) 24 hours post-injection (c) 72 hours post-injection and (d) 5 days post-injection. Each row represents data from a different mouse.

3.2 Dynamic Imaging Results

This study also investigated the short-term dynamic effects over the hour surrounding the first bevacizumab injection. As an example, Figure 2 shows a normalized signal measured for one source and one detector of the collected data acquired for 10 minutes prior to injection and 50 minutes post injection with the duration of the

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injection shown in green. The source detector pair straddled the known tumor location. The acquired signal was filtered using a 300 point moving average filter to remove any respiratory noise from the mouse. Prior to injection there is a stable baseline and then following the injection there is a gradual 30% increase over the baseline signal. We are still investigating a physiological explanation for these early effects.

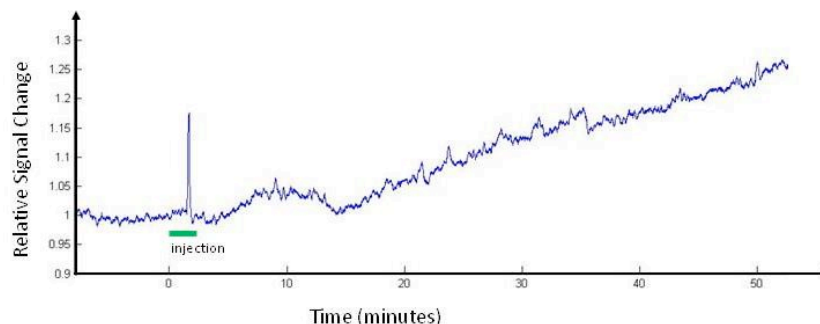


Figure 2. Relative signal change for one source and one detector measurement for 10 minutes pre-injection and 50 minutes post-injection.

4. Discussion and Conclusions

Using optical tomography we were able to study the effects of bevacizumab on tumor vasculature. We examined the effects over a 7 day period and saw a drop in the total hemoglobin over 24-72 hours followed by return of the total hemoglobin levels at 5-7 days. We also looked at the effects over the hour surrounding the initial injection and found that there are some measureable early effects. It appears that optical tomography is highly sensitive to these changes in total hemoglobin and can be used to study early tumor response in drug treatment. In this ongoing study we will continue to explore the time course effect of bevacizumab including its effect on other tumor types.

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