

Optical Tomographic Imaging of Tumor Response to Anti-Angiogenic Drugs in Small Animals

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Abstract— Using diffuse optical tomography we have imaged early vascular responses to anti-angiogenic treatments in a small animal tumor model. Images acquired from 1 to 7 days after drug administration show measurable changes in hemoglobin concentration. There are observable differences between mice treated with anti-angiogenic drugs and mice that receive a placebo treatment.

I. INTRODUCTION

Anti-angiogenic agents have been investigated for use in cancer therapy for the past thirty years. Many of these agents target the growth factors that promote angiogenesis, an important feature of tumor growth. Although the clinical results from many studies involving these anti-angiogenic agents have been promising, there is a large variability in the effectiveness of these agents depending on the type of cancer and even within various trials on the same tumor type [1,2]. The goal of this study is to design a non-invasive method to predict the outcome of the anti-angiogenic therapy as early as possible.

Some studies have observed regression of the existing vasculature as quickly as 24 hours after the anti-angiogenic drug administration [3-6]. Furthermore, preliminary studies on a limited number of mice showed that early changes within 24 hours of drug injection were observable using Optical Tomography (OT) [7]. OT is a non-invasive imaging modality that uses light to measure the levels of oxyhemoglobin (HbO₂) and deoxyhemoglobin (Hb). In this study we employ a Ewing sarcoma tumor model which is known to respond well to treatment with bevacizumab, an anti-angiogenic drug. Using 10 animals we performed OT measurements at 1,3,5, and 7 days after an initial injection with bevacizumab. We demonstrate that OT can detect changes in the vasculature within the first week of treatment.

II. METHODS

A. Experimental Procedure

In this study 10⁶ 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. Control mice were treated with an albumin placebo instead of the bevacizumab. Full optical tomographic data sets were collected prior to the initial drug injection and then 1, 3, 5, and 7 days following the initial injection. Each mouse was positioned in an imaging cylinder with the tumor located between two rings of sources and detectors. The area around the mouse was filled with 1% Intralipid optical matching fluid. The OT data sets were acquired over approximately three minutes (1000 frames collected at >5 frames/second). 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.

B. Diffusion Optical Tomography Imaging

The diffuse optical tomography imaging in this study was performed using a digital optical tomography system previously designed and developed in our laboratory [8]. 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.

C. Image Reconstruction

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 [9] 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.

III. RESULTS

We imaged 8 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 mouse shown in the top row of Fig. 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 [10]. These findings were confirmed with well-established immunostaining methods for endothelial and vascular mural cells, using anti-PECAM-1 and anti- α SMA antibodies.

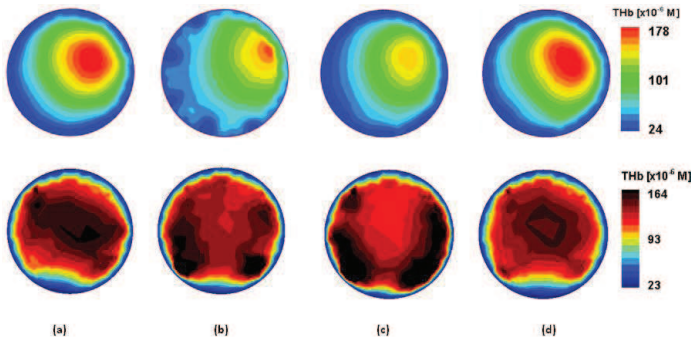


Fig. 1. Total Hemoglobin concentration at four time points (a) prior to injection (b) 24 hours post-injection (c) 72 hours post-injection and (d) 5 days post-injection. The top row contains images from a treated mouse and the bottom row contains images from a control mouse.

As seen in Fig. 1 (bottom) the control mouse that did not receive bevacizumab treatment did not show the strong decrease in total hemoglobin that was seen in the treated mice. The fluctuations in the total hemoglobin in the control mouse are likely due to the continued growth of the tumor with increasing heterogeneity as the tumor size increases.

IV. 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 did not see these effects in the control mice. It appears that optical tomography is highly sensitive to the 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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REFERENCES

- [1] F. Kabbinavar, et al, "Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer," *J. Clin. Oncol.* 21, pp. 60-65 (2003).
- [2] K.J. Kim, et al, "Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo," *Nature* 362, pp. 841-844 (1993).
- [3] J. Huang, et al, "TNP-470 promotes initial vascular sprouting in xenograft tumors," *Molecular Cancer Therapeutics* 3(3), pp. 335-343 (2004).
- [4] N.R. Smith, et al, "Acute pharmacodynamic and antivascular effects of the vascular endothelial growth factor signaling inhibitor AZD2171 in Calu-6 human lung tumor xenografts," *Molecular Cancer Therapeutics* 6, pp. 2198-2208 (2007).
- [5] D.W. Miller, et al, "Rapid Vessel Regression, Protease Inhibition, and Stromal Normalization upon Short-Term Vascular Endothelial Growth Factor Receptor 2 Inhibition in Skin Carcinoma Heterotransplants," *American Journal of Pathology* 167(5), pp. 1389-1403 (2005).
- [6] T. Inai et al, "Inhibition of Vascular Endothelial Growth Factor (VEGF) Signaling in Cancer Causes Loss of Endothelial Fenestrations, Regression of Tumor Vessels, and Appearance of Basement Membrane Ghosts," *American Journal of Pathology* 165(1), pp. 35-52 (2004).
- [7] J. Masciotti et al, "Monitoring tumor growth and treatment in small animals with magnetic resonance optical tomographic imaging," in *Multimodal Biomedical Imaging*, Fred S. Azar, Dimitris N. Metaxas, eds., SPIE International Symposium on Biomedical Optics, Proc. SPIE 6081, #608105 (2006).
- [8] J.M. Lasker et al, "Digital-Signal-Processor-Based Dynamic Optical Tomography Imaging System," *Review of Scientific Instruments* 78(8), 083706 (2007).
- [9] H.K. Kim, A.H. Hielscher, "A PDE-constrained SQP algorithm for optical tomography based on the frequency-domain equation of radiative transfer", *Inverse Problems* 25, 015010 (2009).
- [10] R.T. Tong, et al, "Vascular Normalization by Vascular Endothelial Growth Factor Receptor 2 Blockade Induces a Pressure Gradient Across the Vasculature and Improves Drug Penetration in Tumors", *Cancer Research* 64, 3731-3736 (2004).