

Monitoring of Anti-Angiogenic Drug Response with Dynamic Fluorescence Imaging

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Abstract—We present the results of a study in which we monitored anti-angiogenic therapy response in a mouse cancer model with in-vivo dynamic fluorescence imaging. In the experiments indocyanine green (ICG) was used as a fluorescent optical contrast agent and its time-dependent uptake and washout before and after the administration of bevacizumab was recorded. We found that compared with other organs, tumors showed a noticeable difference in ICG kinetics after treatment.

I. INTRODUCTION

Angiogenesis plays a critical role in the tumor progression, and the inhibition of tumor angiogenesis has shown to be a promising therapeutic strategy for controlling tumor growth [1,2,3]. To date, four anti-angiogenic drugs have been approved for human use by the Food and Drug Administration (FDA). However, the therapies are expensive and effectiveness changes with tumor types. Therefore, non-invasive imaging biomarkers for monitoring angiogenesis and investigating early responses of anti-angiogenic treatment could be useful for deciding the optimal therapeutic regimen for patients[4].

In this study, we investigated the potential of dynamic fluorescence imaging using ICG as a reliable technique for detecting early response of anti-angiogenic treatment.

II. METHOD

A. Contrast Agent

ICG, which is approved by the FDA for human use, has been widely used as an optical contrast agent in the near infrared wavelength range and has been well characterized in many clinical applications. In plasma, ICG binds to albumin almost completely. The effective molecular weight of ICG-albumin binding reaches 67 kDa. This characteristic makes ICG behave like a macromolecular contrast agent in plasma. The uptake slope and the peak value of recorded ICG time traces are correlated with the permeability of the vasculature. It has been shown that ICG shows higher vascular permeability in leaky tumor vasculature than in normal vasculature. In particular, using optical spectroscopy and magnetic resonance imaging, Cuccia et al [5] have demonstrated that a necrotic tumor shows lower peak

concentration and lower permeability of ICG.

B. Tumor Model

10⁶ Tumor cells (SK-NEP1-luc, human Ewing sarcoma), engineered to express bioluminescence, were injected intraperitoneally in 9 NCR nude mice 3-4 weeks prior to treatment and measurement. During this procedure all animals were anesthetized with a mixture of Ketamine (50 mg/kg) and Xylazine (5 mg/kg). Subsequent tumor growth was measured bi-weekly by bioluminescence imaging with a Xenogen IVIS imaging system. Treatment with the FDA approved anti-angiogenic agent bevacizumab commenced when the tumor weight reached approximately 1g.

C. Experiment and Analysis

Bioluminescence and dynamic fluorescence imaging for measuring the bevacizumab treatment response, were performed with a Maestro2 In Vivo imaging system (CRI, Inc., Woburn, MA). For the bioluminescence imaging, we employed a 560 nm (± 10 nm) emission filter set and low-light image mode. For the fluorescence imaging, we employed a 704 nm (± 20 nm) excitation filter set, a 820 nm (± 10 nm) emission filter set, and a 5 frames/second imaging frame rate.

The mice were anesthetized with a gas mixture of 1.5% isoflurane and oxygen. For the injection of the bioluminescence and fluorescence markers, the tail vein was catheterized with a 30 gauge needle and PE10 tubing. To get three different directional views, the mice were placed between two 45-degree angle mirrors in the chamber. Two minutes after a 70 μ L Luciferin IV injection, a bioluminescence image was taken with 3 minutes exposure time. The position of the animals was maintained under the anesthetization for 35 minutes until the fluorescence imaging was completed.

Autofluorescence was measured with the same set up as was used for the dynamic fluorescence imaging. Consecutively, 200 μ M, 60 μ L ICG was injected through the catheter and the dynamic fluorescence imaging started at the time of the first injection. In total, 900 fluorescence images were acquired within 3 minutes. After the baseline

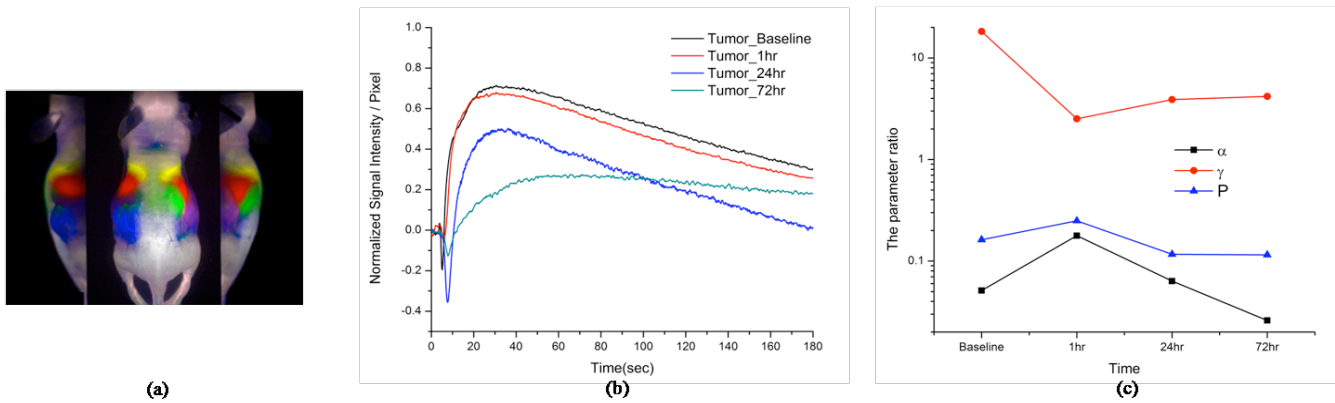


Fig. 1. (a) The color-coded map of organs and tumor based on ICG uptake similarity (blue – tumor, red – livers, yellow – lungs, green – kidney , pink – brain) (b) ICG time traces in tumor at different measurement time points (c) The ICG kinetics parameters ratio between tumor and brain (α (black) : the slope of uptake ($y=\alpha \cdot x + \beta$), γ (red) : the washout time constant ($y=A \cdot \exp(-X/\gamma) + B$), P (blue) : the peak intensity)

measurement, 0.2 mL bevacizumab was administered by IV injection. The experiment was repeated at 1, 24 and 72 hours after the initial treatment with bevacizumab. For the same mouse, one batch of ICG had been used for continuous measurements and pure ICG signal degradation was measured to calibrate signal intensity.

We used the DyCE software developed by Hillman et al [6] to generate a color-coded map of organs and tumor (Fig.1.(a)), based on ICG uptake and washout similarity. To reduce the fluctuation effects during image acquisition, the signal of each imaging frame was normalized by the average signal level of all pixels in each frame. The autofluorescence signal was subtracted from the ICG signal. From these normalized time traces, we extracted α , the slope of the uptake, γ , the washout time constant and P, peak intensity of the ICG kinetics.

III. RESULT

The tumor defined by bioluminescence imaging was well identified with the tumor area in the color-coded map (Fig.1.(a)). However, since the color-coded map was generated based on ICG uptake and washout similarity, it showed a broader tumor area as compared to bioluminescence imaging.

Fig. 1. (b) illustrates ICG time traces in a tumor at different measurement time points. After the bevacizumab treatment, the signal traces (red - 1 hour, blue - 24 hour, green - 72 hour) showed slower uptake and lower peak signal intensity of ICG as compared to the baseline trace (black - baseline). Because the injection timing and hemodynamic status of the animals are not exactly the same at every measurement, we used the ICG time traces of the brain as references to calculate the ratio of extracted parameters. In contrast with the tumor, the brain typically showed similar signal traces for measurements performed at different time points. Fig. 1. (c) shows the ratios of tumor and brain ICG uptake slopes, washout time constants and peak intensities. 24 and 72 hours after the bevacizumab treatment, measurements showed a decrease of the ratio in uptake slopes and peak intensities and an increase of the ratio in washout time

constants as compared to the 1 hour points. This observation can be explained by decrease in the permeability of tumor vasculature, which is in agreement with the study of Cuccia et al [4] and Tong et al [7].

IV. CONCLUSION

We demonstrated that dynamic fluorescence imaging can be used to non-invasively monitor early responses to anti-angiogenic drug treatment. ICG was injected intravenously into mice bearing Ewing sarcomas. The dynamic fluorescence signal changes in the tumor were measured 1h, 24 h, and 72h after bevacizumab treatment. An analysis of ICG kinetics showed that the slope of the uptake and peak intensities of time traces in tumors decreased noticeably after the treatment.

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