

ASSESSING VASCULAR TARGETING AGENTS USING DYNAMIC FLUORESCENT CONTRAST *IN VIVO*

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INTRODUCTION

Dynamic contrast enhanced (DyCE) fluorescent imaging was recently demonstrated for identifying the organs in mice based on principal component analysis of contrast kinetics [1]. It occurred to us that this approach could be used to evaluate acute effects of vascular disrupting agents (VDAs), since these cause massive vascular shutdown [2]. As proof of principle, we have now examined the action of Combretastatin A-4 Phosphate (CA4P, Fig.1) on MCF7 human breast tumors growing in nude mice.

METHODS

- MCF7 tumors were implanted in the thigh and allowed to grow to about 1 cm in diameter.
- Mice were anesthetized and 50 μ l indocyanine green (ICG; 260 μ M) was administered as a bolus by tail vein injection. The fluorescence time course was acquired over 230 s using a CRi Maestro small animal fluorescent imaging system.
- CA4P was then administered IP (150 mg/kg in saline 100 μ l) and DyCE repeated following administration of fresh ICG 2 h later. Measurements were repeated after 24 h. As control 100 μ l saline was injected into additional mice.
- For the DyCE study, 5 images were captured per second, exposure time of every image was 50 ms, total time was 200 s using red filter excitation 671-705 nm and detection with 750nm longpass filter.

RESULTS

- As expected Principal Component Analysis of the ICG pharmacokinetics revealed tumor and tumor vasculature as distinct from other tissues (Figure 2, 3)
- Two hours after administration of the VDA CA4P, the perfusion of the tumor was severely reduced and the intensity of fluorescence diminished. (Figure 2-4).
- After 24 h there was considerable recovery in tumor signal.
- Control mice treated with saline showed little variation in perfusion during 3 DyCE scans over 24 h (Figures 5, 6)

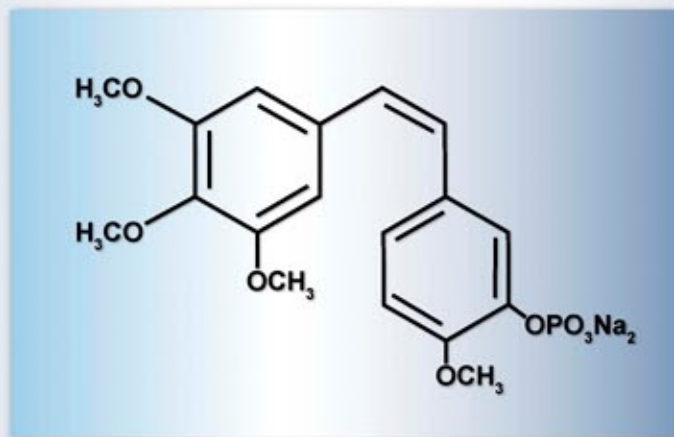


Figure 1: Structure of the Combretastatin A-4P (CA4P)

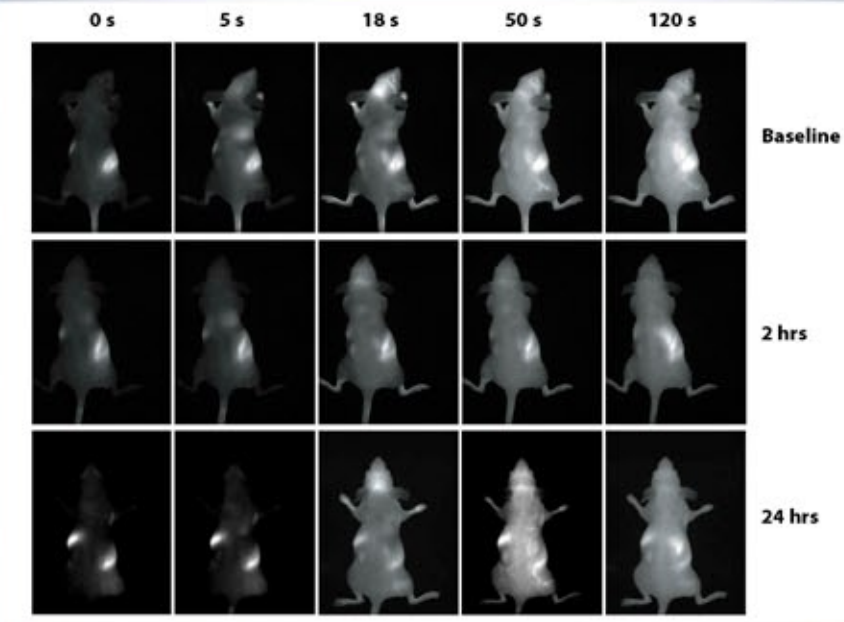


Figure 2: Upper row of images shows fluorescence intensity at various times after infusion of ICG IV in the tail vein. Middle row was obtained 2 hrs after administration of Combretastatin (150 mg/kg in saline 100 μ l IP). Bottom row. Repeat imaging after 24 hrs.

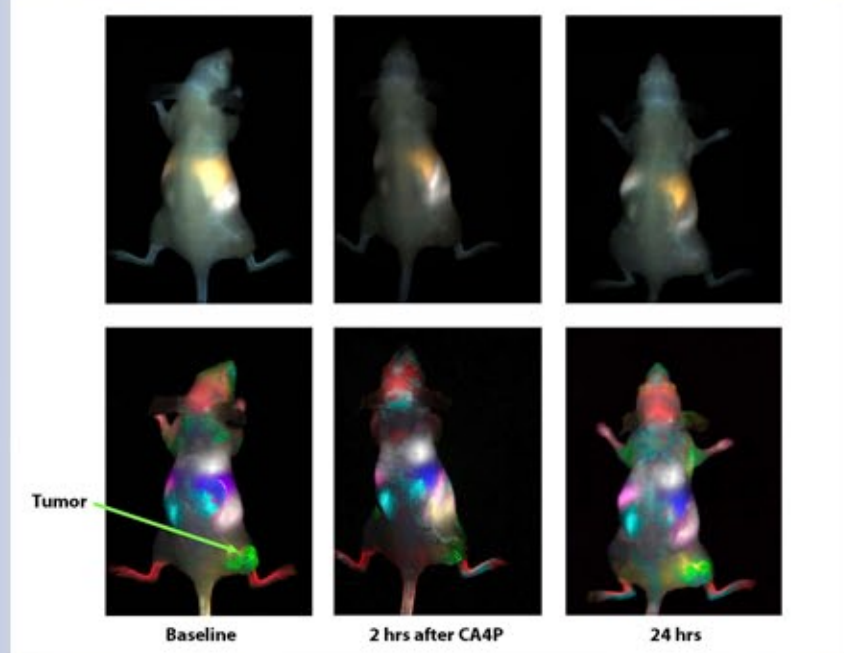


Figure 3: Principal component analysis of mouse organs based on DyCE with respect to CA4P treatment. Multiple organs such as liver, kidneys, primary vasculature and tumor are discriminated.

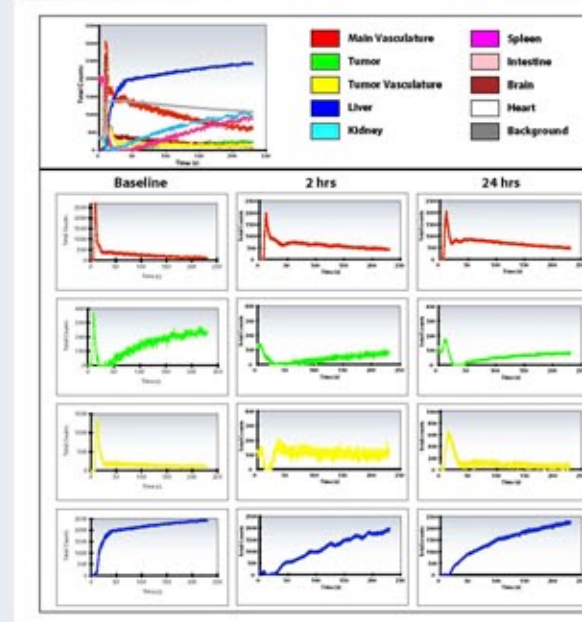


Figure 4: Dynamic Fluorescent Imaging of ICG infusion. Curves show differential variation in tissue signal intensity over a period of 230 s following ICG infusion. Signal in the tumor and vasculature supplying the tumor appear severely diminished following CA4P administration. The nature of the bolus input function is obvious from the primary vasculature.

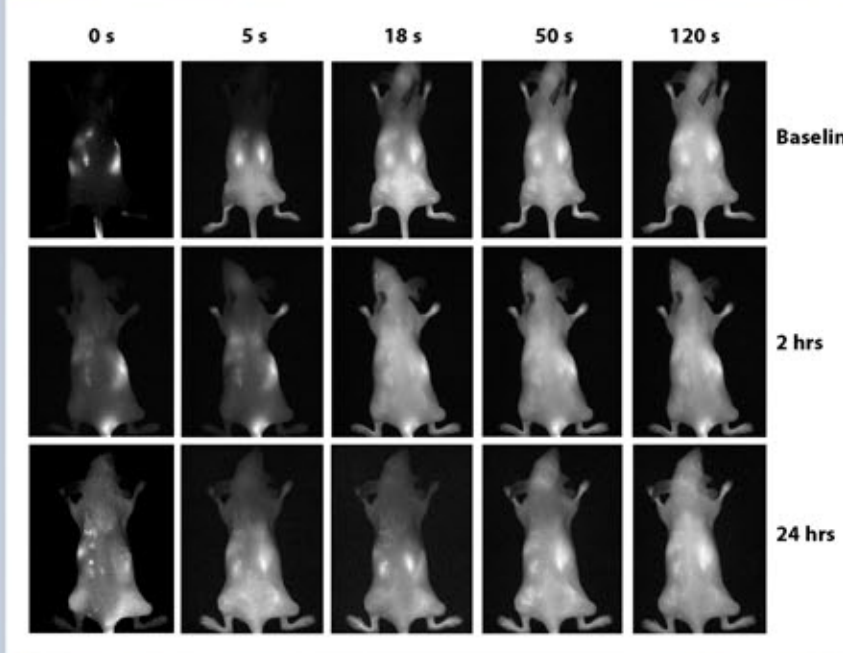


Figure 5: Dynamic Fluorescent Imaging of ICG infusion tumor response to control saline at different time points. Data may be compared with Figure 2, showing relatively consistent behavior over three control scans with respect to saline administration.

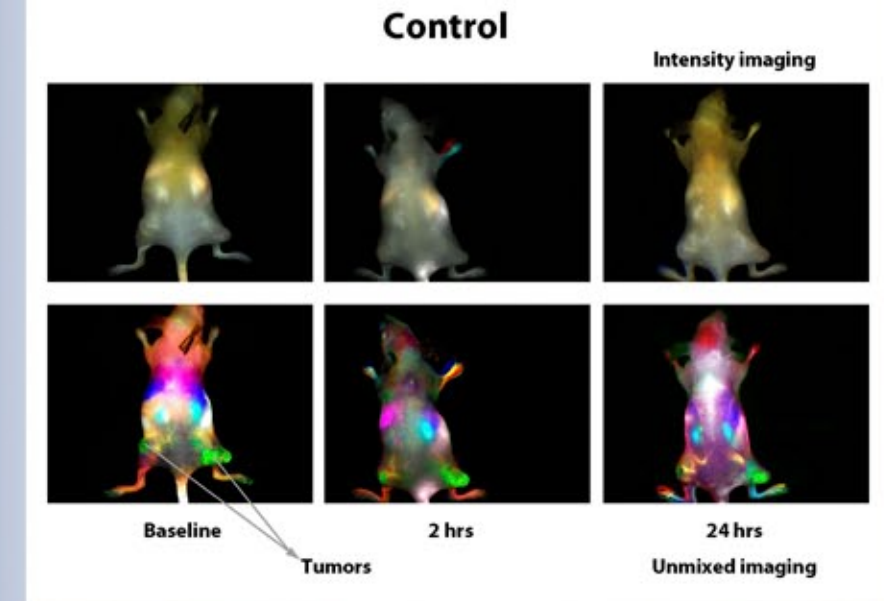


Figure 6: Principal component analysis of mouse organs based on DyCE with respect to control saline treatment

CONCLUSIONS

The method is analogous to one reported recently, which used dynamic bioluminescence imaging to probe the acute effects of VDAs [3]. DyCE adds to the armamentarium of the imaging scientist to probe tumor vasculature non-invasively. It has the advantage of avoiding the need for transfected luciferase-expressing cells. Optical imaging is relatively cheap, easy to implement and allows high throughput. We believe it holds potential for evaluating and optimizing therapeutic drug doses and combinations.

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