

Changes in tumor perfusion and oxygenation following CA4P administration

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Target audience: MR scientists who are interested in oncology, tumor hypoxia and cancer therapies (antivascular and antiangiogenic).

Introduction: Vascular disrupting agents (VDAs) are quite recent anti-cancer drugs targeting the immature and rapidly proliferating endothelial cells of existing tumor blood vessels¹. As the lead compound of this agents' class, Combretastatin A4 phosphate (CA4P) is undergoing a phase III clinical trial. Rapid tumor blood flow shutdown has been also demonstrated in preclinical models and patients by various techniques including dynamic contrast enhanced MRI following CA4P infusion². The induced hypoxia by VDAs (e.g. CA4P) has been also investigated recently in a preclinical model³. The present work correlates a follow-up on two tumor models of both tumor oxygenation and tumor hemodynamics as well as an early monitoring of VDAs effects after CA4P administration. Mapping of tumor oxygenation was assessed with 'MOBILE' (Mapping of Oxygen By Imaging Lipids relaxation Enhancement) a non-invasive MRI method based on the changes in the relaxation properties of the tissue lipids protons⁴, in order to evaluate the ability of the method to follow a decrease in oxygenation. The actual pO₂ decrease was measured by EPR oximetry. Tumor hemodynamics (K^{trans} and vp) variations were finally evaluated by DCE-MRI on same tumor models after the CA4P administration and compared to those obtained within a control group (DMSO injection).

Methods:

Mammary tumor models: NT2 and human MDA-MB-231 tumor cells were implanted whether subcutaneously or at orthotopic site in FVB/Nrj and NMRI nude mice respectively.

Protocol: Hemodynamic parameters and oxygenation maps were obtained for both tumor models at baseline and 3 hours after CA4P administration (100 mg/kg) as well as pO₂ measurements. Different mice cohorts were used for (i) DCE-MRI (ii) MOBILE experiments and (iii) EPR oximetry.

DCE-MRI: Experiments were performed with a 11.7T (Bruker, Biospec) and a quadrature volume coil. T1 weighted gradient echo images were obtained with a fast low angle shot sequence with the following parameters: TE/TR/FA/matrix/bandwidth/zero fill acceleration factor = 2.074ms/15.000ms/40°/128x64/100kHz/1.4. A first set of 400 scans with temporal resolution of 1.19s was acquired with gadoterate meglumine manually administered intravenously after the 20th scan over 2s (0.171 and 0.260 mmol/kg Gd for MDA-MB-231 and NT2 tumors respectively). The washout was then monitor with a slower data set acquired with temporal resolution of 10.1s.

'MOBILE': Experiments were performed with the same preclinical MRI scanner using a surface coil cryoprobe. A segmented IR FISP (Inversion-Recovery Fast Imaging with Steady state Precession) sequence (SSFP FID mode) was used to acquire parametric images of T₁ relaxation time. Acquisition parameters were TR/TE/FA/BW/matrix = 4 ms/1.2ms/5°/100kHz/64x64, 4 segments, and a total acquisition time of 1min20s. The difference in Hertz between water and lipid peaks was evaluated on a single pulse spectrum. These offsets were then used as an imaging frequency offset in the same IR FISP protocol and the water signal was spoiled.

EPR experiments: A 1.1 GHZ in vivo L-band EPR Magnetech system was used 24h after injection of a paramagnetic oxygen reporter probe.

Results: Mean values of R₁ were calculated for each tumor on parametric maps provided by MOBILE at baseline and 3 hours after the CA4P injection. Individual variations of R₁ point out a systematic decrease in tumor pO₂ for both tumor models (Fig.1). This reflects the decrease in actual pO₂ values measured by EPR oximetry. Indeed, actual pO₂ varies from 3.402±1.683mmHg at baseline to 2.740±1.454 mmHg 3 hours after CA4P administration for NT2 tumors and from 10.04±5.351 mmHg at baseline to 7.6±4.593 mmHg for MDA-MB-231 tumors. Considering pooled R₁ values of both models, we observe a significant variation of Lipids R₁ values after the hypoxic challenge (p=0.0020)(Fig.2). Hemodynamics evolution induced by the hypoxic challenge support those outcomes: in both models, relative changes in K^{trans} (difference between post- and pre-treatment) are significantly different between control (DMSO injection) and treated groups for both tumor models (p=0.0051, and p=0.0026 for NT2 and MDA-MB-231 respectively)(Fig.3). A decrease in vp is also observed in treated group compared to control group, although not significant (p=0.1240, and p=0.0762 for NT2 and MDA-MB-231 respectively) (Fig.4).

Figure 1

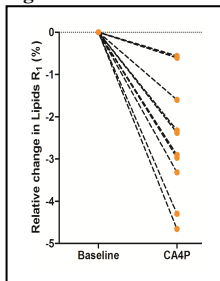


Figure 2

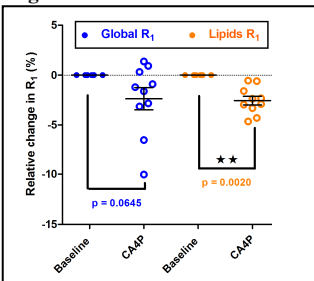


Figure 3

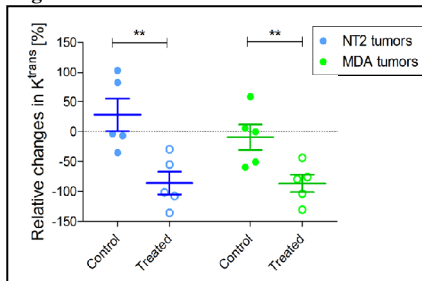
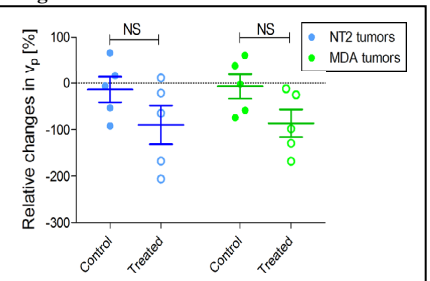


Figure 4



Discussion: CA4P compromises the tumor existing vasculature. Our results demonstrated that within the 3 hours after CA4P administration, the drug induces enough vasculature damage to significantly decrease K^{trans} in two tumor models. EPR oximetry has confirmed the consequent increased hypoxia. The MOBILE technique was also able to follow the decrease in tumor pO₂. Since the MOBILE sequence is also implemented in the clinical setting, it appears as a valuable tool to monitor variations of oxygenation after anti-cancer treatment.

References: 1. Siemann DW et al. *Cancer Treatment Reviews* 2011;37:63-74 2. Nagaiah G et al, *Future Oncol.*, 2004;6:1219-1228
 3. Iversen AB *Acta Oncologica* 2013;52:1320-1326 4. Jordan BF, et al. *Magn Reson Med.* 2013;70:732-744