

MRI evaluation of tumor physiological response to combretastatin A4 phosphate: correlation with a combined radiation response

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Introduction: The vascular targeting agent, combretastatin A-4-phosphate (CA4P) causes tumor vascular shutdown inducing massive cell death. Although massive necrosis can be induced, tumors usually regrow from a thin viable rim. Thus, a combination of VTAs with additional conventional therapeutic approaches, e.g., radiation, will be required (1). For combination with radiotherapy, measurement of tumor oxygen dynamics will be especially important, since reduced perfusion can induce hypoxia, potentially modulating radiation response. Thus, we have assessed dynamic changes in tumor oxygenation as compared with vascular perfusion/permeability after CA4P treatment by combining ¹H and ¹⁹F MRI (2). Based on pathophysiological changes monitored by MRI, optimum scheme of the combined radiation and CA4P treatment was designed and experimental treatment was initiated on rat breast tumors.

Materials and Methods: Rat mammary carcinoma 13762NF was implanted syngeneically in a skin pedicle surgically created on the foreback of Fisher 344 adult female rats and allowed to grow to ~ 1 cm diameter.

MRI study: MRI studies were performed using a 4.7 T Varian Inova imaging system. Each rat was maintained under general anesthesia (air and 1% isoflurane). A tunable (¹H/¹⁹F) volume RF coil was placed around the tumor-bearing pedicle. ¹H MRI R₂^{*} maps were obtained before and 2 h after CA4P (30 mg/kg, i.p., OXiGENE, Inc.) by gradient echo sequence (GEMS) with 8 echoes (TR = 195 ms, TE = 7 ms and spacing = 6 ms). Dynamic contrast enhanced (DCE) MRI using a T₁-weighted spin echo sequence (TR = 70 ms, TE = 12 ms) based on i.v. bolus injection of Gd-DTPA-BMA through a tail vein catheter was also acquired before, 2 h and 24 h after CA4P. For ¹⁹F NMR oximetry, hexafluorobenzene (50 μl) was injected directly into the tumor along two or three tracks in a single central plane of the tumor using a fine sharp needle (32G), as described in detail previously (25). **FREDO**M (Fluorocarbon Relaxometry using Echo planar imaging for Dynamic Oxygen Mapping) MRI was performed to acquire a series of pO₂ maps under air or oxygen breathing before and at different time points after CA4P. Data analysis was carried out on a voxel-by-voxel basis with IDL based house made software.

Experimental treatment: Animals bearing 13762NF tumors (n = 30) were grouped as: 1) control without treatment; 2) CA4P (30 mg/kg, i.p.) alone; 3) Radiation (IR) alone (5 Gy single dose); 4) IR (5 Gy) + CA4P (1 h post IR, 30 mg/kg, i.p.); 5) CA4P (30 mg/kg) + IR plus O₂ (24 h post CA4P, 5 Gy). The animals started to breathe oxygen (100% O₂ + 1% isoflurane) 20 min before receiving a 5 Gy IR delivered by Accuray system.

Immunohistochemistry: Immunostaining for Hoechst 33342 (perfusion marker) and CD31 (vascular endothelium) was performed to correlate with imaging findings.

Results: ¹H MRI showed that tumor blood perfusion/permeability by DCE MRI decreased significantly to ~30% of baseline pretreatment level at 2 h after CA4P (30 mg/kg, i.p.) infusion, which recovered fully after 24 h in a thin peripheral region, but not the tumor center. Analysis of R₂^{*} maps revealed significantly increased values after 2 h, compared to pretreatment (88.6 v.s. 85.1 s⁻¹, p < 0.05). Tumor pO₂ by ¹⁹F MRI was found to decline within 60 min, become significantly lower at 90 min, and decrease further at 2 h after CA4P infusion. At this time there was no response to breathing O₂. Some regional recovery was seen 24 h later, but the pO₂ was still significantly lower than the pretreatment level. However, oxygen breathing at 24 h point modulated tumor pO₂ significantly, which resulted in essential elimination of tumor hypoxia (Fig. 1). Correlating well with MRI observations, the tumors of Group 5 with radiation plus oxygen 24 h post CA4P showed a significantly prolonged growth delay (* p < 0.05, Fig. 2). Histological data using the perfusion marker, Hoechst 33342, confirmed a significant decrease in perfused vessels 2 h after CA4P, while there was recovery evident at 24 h.

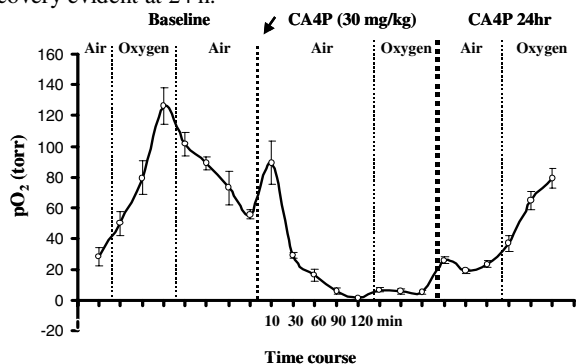


Fig. 1. Tumor pO₂ monitoring of tumor oxygen dynamics in response to CA4P

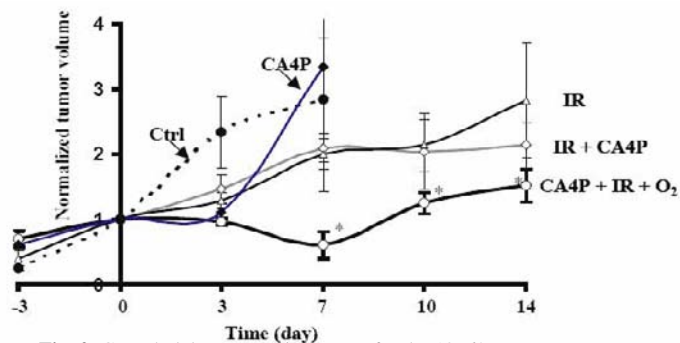


Fig. 2. Growth delay versus time curve for the 13762NF tumors.

Discussion: There is a distinct similarity between the results of the pO₂ measurements and the more traditional DCE, but the quantitative pO₂ values provide the potential for exploiting synergy with other oxygen dependent therapies. The observations further demonstrate the value of **FREDO**M in assessing dynamic changes in regional tumor pO₂ *in vivo* in response to intervention. We believe that dynamic measurements are particularly valuable for understanding the mode of action of therapeutic response to VTAs. Most significantly, these measurements lay a foundation to optimize the timing of combination therapy involving fractionated radiotherapy and multiple doses of VTAs.

- References:**
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