HRMAS 1H spectroscopy of the novel VEGF-RTK inhibitor AG013736 in a breast cancer model

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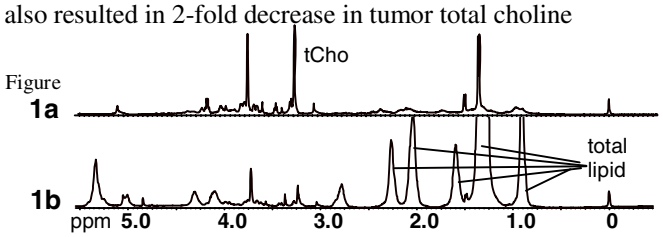
Introduction: AG013736, a novel and potent inhibitor of the VEGFR/PDGFR tyrosine kinases has shown broad anti-tumor activity in xenograft models of cancer [1]. DCE-MRI studies in a breast cancer model have demonstrated a significant decrease in tumor endothelial transfer constant (K^{ps}) with AG013736 [2]. This study utilized HRMAS ^{1}H spectroscopy to evaluate potential AG013736-induced changes in breast tumor metabolism that may be associated with treatment response. The identification of metabolic bio-markers for response to anti-angiogenic therapy may aid in designing future clinical spectroscopy studies.

Methods: Female nude mice were implanted with the human breast cancer line BT474, a Her2/neu overexpressing variant. Treated mice (n=4) received 25 mg/kg i.p. b.i.d., of AG-013736 while control mice (n=5) received an equivalent volume of vehicle. After 7 days of treatment mice were euthanized and tumor tissue was collected, flash frozen and stored at -80 °C. Ten to twenty milligrams of tumor tissue was placed in a zirconium rotor with 3 microliters of D_2O with TSP. HRMAS ^{1}H spectra were acquired using a Varian Inova 500 MHz spectrometer equipped with a gHX gradient nanoprobe, at 1°C. The samples were spun at 2250Hz, at the magic angle (θ=54.7°). One- and two-dimensional ^{1}H HRMAS spectra were obtained using the standard one pulse sequence, and a rotor synchronized adiabatic TOCSY pulse sequence. Metabolite peak areas were identified using one- and two-dimensional spectra and quantified using ACD 8.0 (Advanced Chemistry Labs, Inc; Toronto, Canada). Metabolite levels were reported as ratios of peak areas. Total lipid (sum of 5 major lipid peaks, Fig 1b), total choline (sum of free choline, glycerophosphocholine and phosphocholine) and and taurine concentrations in tumors were calculated.

Results: Representative ^{1}H HRMAS spectra from a control and an AG013736-treated tumor are shown in Figure 1a and 1b respectively. A 3.5-fold increase in mean tumor total lipid was observed in treated tumors relative to controls (Figure 2). AG013736 treatment also resulted in 2-fold decrease in tumor total choline (tCho) to creatine ratio ($p<0.05$) and a 1.3 fold decrease in taurine (Tau) to creatine ratio (Figure 3). Two-dimensional spectroscopy revealed that average phosphocholine, choline and glycero-phosphocholine were reduced in treated tumors compared to controls.



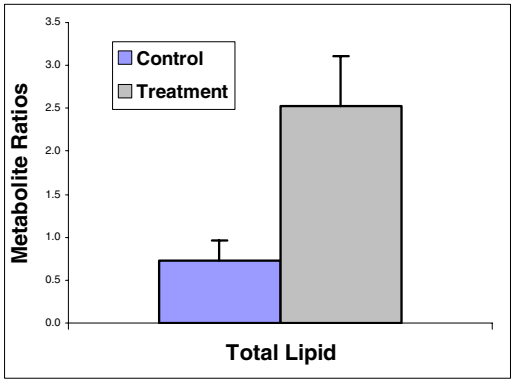


Figure 2 (Mean + SEM)

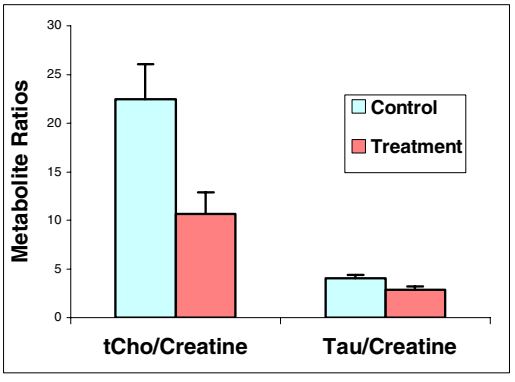


Figure 3 (Mean + SEM)

Discussion and Conclusions: ^{1}H HRMAS spectroscopy showed that treatment with AG013736 resulted in a large increase in tumor lipid signal which may reflect apopotosis or necrosis [3,4]. In addition the decrease in total choline is consistent with other proton spectroscopic studies of response to anti-cancer agents that work via different mechanisms [5]. The results indicate ^{1}H HRMAS spectroscopy may provide additional biomarkers of treatment response for anti-angiogenic therapies such as the VEGF/PDGFR TK inhibitor AG013736.

References: [1] Hu-Lowe et al., Proc. AACR 2002; #5357. [2] Wilmes et al., Proc ISMRM 2003 #1243. [3] Hakumaki JM, Kauppinen R, TIBS 2000. [4] Delikatny EJ et al. CanRes 2002. [5] Glunde K, et al. Cancer Res 2004.

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