Effect of c-Myc Expression on Cellular-Interstitial Water Exchange Kinetics: Conditional Transgenic Mouse Breast Cancer Model

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Introduction: Dynamic contrast-enhanced (DCE)-MRI uses the concentration-time curve of a paramagnetic contrast agent (CA), gadodiamide here, for quantitative estimation of vascular permeability, particularly in assessing response to antiangiogenic therapy in breast cancer[1, 2]. Transgenic MTB/TOM mice conditionally express the human oncogene c-myc in mammary glands in response to doxycycline (dox) treatment[3]. Dox withdrawal leads to tumor disappearance in 80% of animals after 2-4 weeks[3]. Also, PET studies have shown that after 96 hrs of de-induction by dox withdrawal, more than 50% reduction of FDG uptake is observed in this model[4]. The Shutter-Speed Model[5] (SSM) (also called BOLERO)[5] differs from the Standard model (SM) of DCE-MRI pharmacokinetics in allowing finite equilibrium transcellular water exchange kinetics. The SM assumes these effectively infinitely fast. Using SSM, Yankeelov *et al.*[5] have demonstrated that the Fast-Exchange-Limit (FXL) SM assumption can lead to systematic inaccuracies in pharmacokinetic parameter estimates. In this study, we evaluated the effect on cellular-interstitial water exchange in this mouse breast cancer model using DCE-MRI data measured with dox and 4 days after withdrawal of dox and analyzed with the SSM formalism.

Material and Methods: Starting at the age of 6 weeks, the MTB/TOM mice (n = 3) were treated with dox 2 mg/mL in their drinking water. The administration of dox induced c-myc oncogene expression in a mammary-specific manner. Palpable tumors were found approximately 15 weeks after induction. MR experiments were performed at 4.7 Tesla when the tumor size reached about 8-10 mm in diameter, just before and 96 hrs after, withdrawal of dox. The precontrast tissue T_1 relaxation time was measured using the TOMROP sequence[6] (*i.e.*, TI = 480 ms (4 heartbeats)), number of TI intervals = 30, flip angle (θ) = 10°, FOV = 25 mm^2, matrix size = 128 X 64, and thickness 1.5 mm) as describe elsewhere[6]. Three hundred (NA =1), low-resolution (16 phase encodes) images were acquired from the same slice using an ECG-gated saturation-recovery GRE sequence (TE = 2.2 ms, TR = 6 ms, θ = 90°, matrix size = 128 x 16) with a temporal resolution of about 2 s/image[6]. A 100 μL bolus of 10 mM gadodiamide (Omniscan, Nycomed, Princeton, NJ) in saline was injected intravenously after acquiring 12 pre-contrast images. An arterial input function (AIF) was obtained from the left ventrical lumen using a baseline blood R_1 = 1.7± 0.34 s and hematocrit (Hct = 0.5) [$R_1 \equiv T_1^{-1}$]. The longitudinal CA relaxivity for blood and interstitial space was set to r_1 = 4.0 mM^{-1}s^{-1}. The mean R_{10} value for the Region-of-Interest (ROI) was 1.6 ± 0.24 s. The ROI data were fitted using an AIF (**Fig.1**) with the SM and the SSM to calculate SM K^{trans} and SM v_e [the extracellular volume fraction], and SSM K^{trans}, SSM v_e, and the intracellular water molecule lifetime (τ_i), respectively. All image reconstruction and data analysis were performed using an in-house software package written in MatLab (v. R2011b; Math- Works, Natick, MA). In the present study, a non- linear least square fitting method was used, and the sum of squared residuals served as a measure of fitting goodness. Data are expressed as mean (± standard deviation). Differences were examined by paired t-tests, and significance was inferred at $p \leq 0.05$.

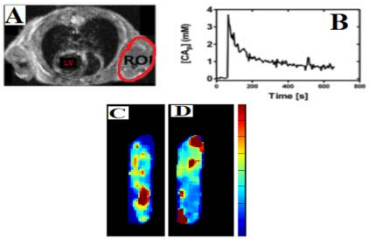

Fig.1. **A**.T_2 weighted scout image with left ventricle (LV) and tumor Region of Interest (ROI). **B**: AIF curve obtained from the LV lumen. **C**. 4 days post-dox K^{trans} and **D.** τ_i maps. The range for K^{trans}: 0-0.5 [min^{-1}] and τ_i = 0-2 [s].

Results: A typical T_2-weighted scout image with ROIS in the left ventricle and ROIs shown in in Fig.1A. The mean correlation coefficients (R^2) for SSM fit were about 0.85, respectively. From ROI analysis, SM K^{trans} with dox (mean K^{trans} = 0.27 ± 0.077: [min^{-1}]) was not significantly different (p = 0.07) from SM K^{trans} without dox (mean K^{trans} = 0.12 (± 0.02): [min^{-1}]). In contrast (Fig. 2), SSM K^{trans} with dox (mean K^{trans} = 0.20 ± 0.076: [min^{-1}]) and without dox (mean K^{trans} = 0.13 ± 0.02: [min^{-1}]) showed a significant difference (p = 0.034). Estimates of τ_i with dox (mean τ_i = 1.1 ± 0.11: [s]) and without dox (mean τ_i = 0.57 ± 0.27: [s]) were significantly different (p = 0.035).

Discussions: SM estimates of K^{trans} and v_e showed a decreasing trend with withdrawal of dox but was not statistically significant. The SSM estimated mean K^{tans} and v_e are larger than means estimated by the SM, but the SSM can differentiate the switching off and on of the c-myc oncogene by administration or withdrawal of dox. These observations and the estimated parameter values are in good agreement with previously reported observations from animal studies. Our preliminary results demonstrate that the mean lifetime of intracellular water (τ_i) can serve as a marker of metabolic changes in breast cancer. Since τ_i is an inverse measure of metabolic activity[7], these results suggest slower metabolic fluxes during doxycycline induction of c-myc. It is interesting that τ_i increased after therapy on a different murine (c-Myc-driven) spontaneous breast cancer model.[9]

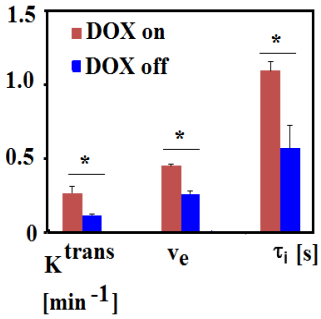

Fig.2. Mean DOX on and off of SSM parameters. The error bars indicate SEM. * represent statistically significant.

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