Acute changes in cellular-interstitial water exchange rate in DB-1 Melanoma xenografts after lonidamine administration as a marker of tumor energetics and ion transport

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Introduction: The analysis of DCE-MRI data using an SSM, measures differences in the water-exchange rate between the extracelluar and intracellular compartments yielding three parameters, the mean lifetime of intracellular water protons, τ_i, the mass-transfer constant for transfer of the contrast agent between the vasculature and interstitium K^{trans}, and the interstitial volume fraction, v_e (1). A recent study has shown that τ_i is inversely correlated with ATP concentration in yeast cell suspensions (2). However, this relationship needs to be validated *in vivo* as the host tumor micro-environment plays a major role in vascular hemodynamic measurements. We have recently reported that acute changes in melanoma xenograft intracellular pH (pHi) and bioenergetics (NTP/Pi) occur 40 minutes after administration of lonidamine (LND), a small molecule that inhibits the monocarboxylate transporter-1 (MCT-1) (3). Thus, the purpose of this study was to evaluate whether the changes in ATP levels correlated with changes in tumor τ_i after LND administration in DB-1 human melanoma xenografts.

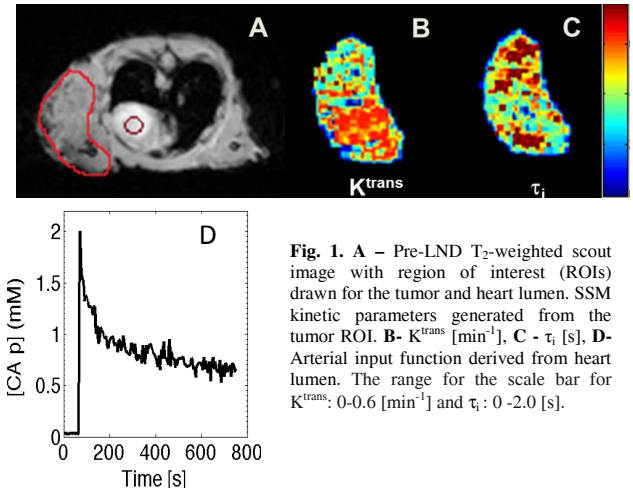

Fig. 1. A – Pre-LND T_2-weighted scout image with region of interest (ROIs) drawn for the tumor and heart lumen. SSM kinetic parameters generated from the tumor ROI. **B-** K^{trans} [min^{-1}], **C -** τ_i [s], **D-** Arterial input function derived from heart lumen. The range for the scale bar for K^{trans}: 0-0.6 [min^{-1}] and τ_i : 0 -2.0 [s].

Materials and Methods: In order to simultaneously measure the arterial input function (AIF) and contrast agent (CA) concentration of tumors, one million DB-1 cells were injected subcutaneously into the nude mice (n = 6) dorsal to the heart. Melanoma xenografts were allowed to grow until they reached 10-15 mm in diameter along the longest axis of the tumor. MR studies were performed on a 9.4 T/31 cm horizontal-bore Varian system using a 35 mm inner diameter transmit-receive volume coil (M2M). Mice were anesthetized using 1% isoflurane in oxygen administered through a nose cone. A rectal thermistor and respiration pillow were placed and connected to a small animal vital sign monitor that detected core body temperature and respiration. The precontrast relaxation time constant, T_{1_0}, was measured using the T_1 by Multiple Read Out Pulses (TOMROP) sequence (4): TR/TE/θ/FOV/matrix/NEX/slice/thickness = 6 ms/2.2 ms/100/128 x 64/40 mm^2/2/1 mm, TI (inversion time) = 4 heartbeats (480 ms), number of TI intervals = 40. The DCE-MRI protocol used an ECG-gated T_1-weighted saturation-recovery GRE sequence (4) with TR/TE/θ/FOV/matrix/NEX/slice/thickness = 7 ms/2.75 ms/900/35 mm^2/128 x 32/2/1 mms with ts = 1 heartbeat (120 ms) to obtain 150 serial images from the same slice with a temporal resolution of about 5 s/image. Gd-DTPA was administered by tail vein injection at a dose of 0.1 mmole/kg of body weight. DCE-MRI was performed after 40 minutes of LND injection, while keeping the mouse in the magnet so that data from the same imaging slice could be acquired again. The longitudinal relaxivity of a CA for blood and interstitial space was set to $r_1 = 4.0\ mM^{-1}s^{-1}$. An AIF was derived from the left ventricular lumen (**Fig. 1A**) (4) using a baseline blood $T_{1_0} = 3.3$ s and hematocrit (Hct=0.45). All image reconstruction and data analyses were performed with an in-house software package written in MatLab (v. R2012b; Math- Works, Natick, MA). A non-linear least squares fitting method was used and the sum of the squares served as a measure of the goodness-of-fit. The kinetic model parameters were calculated before and after LND administration. The significance of changes in these parameters was tested using a paired t-test, and significance was inferred at $p \leq 0.05$.

Results: A typical T_2 -weighted pre LND scout image with ROIs (tumor and heart lumen) are shown in **Fig. 1A**. The mean T_{1_0} value for tumor ROIs was 3.0 ± 0.16. **Figure 1B and C** show the pre-LND SSM K^{trans} and τ_i maps, while **Fig. 1D** shows a representative plot of the AIF from this animal. A significant increase in τ_i values was noted after LND injection (1.02 ± 0.43 [s]) versus 2.34 ± 1.34 [s], p = 0.04). A significant decrease in SSM K^{trans} and v_e was observed after LND administration. The mean K^{trans} decreased from 0.32 ± 0.19 [min^{-1}] to 0.18 ± 0.06 [min^{-1}] (p <0.02, **Fig. 2A**), while the mean v_e decreased from 0.65 ± 0.08 to 0.42 ± 0.08. The percent changes from baseline to post-LND are shown in **Fig. 2B**. The mean correlation coefficients (R^2) for SSM fit were about 0.85

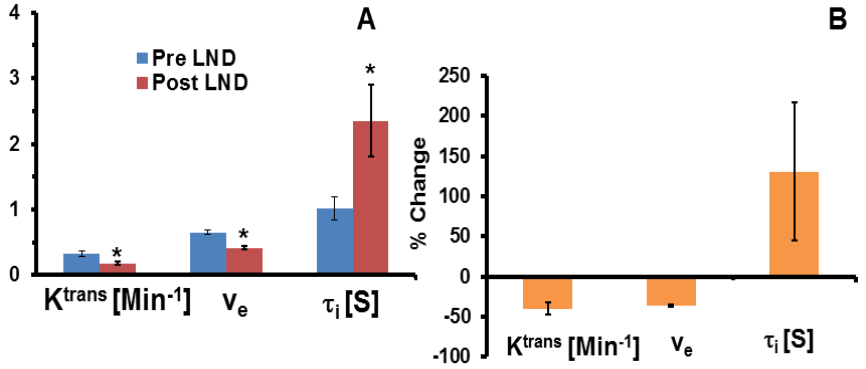

Fig. 2. A. Mean pre- and post-LND SSM parameters. **B**. % Change in K^{trans}, v_e and τ_i. K^{trans} decreased by 40.1±7.0%, v_e decreased by 36.0±1.40%, whereas τ_i increased by 130.9±86.2% following LND administration. The error bars indicate SEM. * represent statistically significant.

Discussion and Conclusion: Generally, an increase in τ_i is associated with a change in cell volume, membrane permeability or ion transport as τ_i is related to the cell size [τ_i= (v/PA)], where v is the volume of the cell, P is the permeability of the cell membrane, and A is the surface area of the cell (1). A decrease in cell as well as vascular permeability probably occurs after LND administration since we observed a significant drop in K^{trans}. A significant decrease in tumor ATP following LND has been reported earlier by Floridi and Lehninger (5) on the basis of polarographic data on isolated mitochondria from Ehrlich ascites tumor cells. Alternatively this ATP decrease could result from inhibition of pyruvate transport into mitochondria, resulting in substrate deprivation for the tricarboxylic acid cycle. The latter explanation is simpler and appears to be more plausible in view of the demonstration by Spencer and Lehninger (6) that another MCT inhibitor, α-cyano-4-hydroxycinnamate, blocks the transport of lactate out of the plasma membrane and also inhibits transport of pyruvate through the mitochondrial membrane. Thus, it appears that these changes in metabolic fluxes of lactate and pyruvate produce an increase in τ_i (4). The increase in τ_i is probably associated with reduced bioenergetics as ATP is required for membrane ion pump activity and the development of the primary ion gradient, which powers secondary active symporters and antiporters. Transmembrane cation flux appears to be linked to water transport by a mechanism that remains to be delineated. Our study shows that changes in K^{trans} and τ_i might serve as surrogate markers of cellular energy status and transmembrane water exchange or cycling While standard model (SM), DCE-MRI, only measures tissue vascular hemodynamics, analysis with the SSM model provides a sensitive method for imaging tumor energetics and membrane transport. Future studies will assess the inherent heterogeneity of the cell permeability and ion-transport and correlate with tumor hypoxia as a meaningful biological marker of tumor diagnosis and response assessment markers.

Acknowledgements. NIH grants 5R01CA129544-04, 1RO1CA172820-01A1. **References:** (1). Yankeelov TE et al. Magn Reson Med 50(6):1151-1169, 2003. (2). Zhang Y et al. Biophysical Journal 101:2833-2842, 2011. (3). Nath K, et al. NMR Biomed 26(1):98-105, 2013. (4). Zhou R et al. Magn Reson Med 52(2):248-257, 2004. (5). Floridi A, et al. Arch. Biochem. Biophys 226(1): 73–83, 1983. (6). Spencer TL et al. Biochem. J 154(2): 405–414, 1976.