Non-invasive quantification of intracellular redox state in tissue by hyperpolarized ^{13}C-NMR

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Introduction

Redox state is a key mediator for many biological processes including cellular metabolism, growth, differentiation, gene transcription and signaling.[1-4] Including the NAD-coupled redox states in mitochondria and cytosol, abnormal cellular redox states have been linked to pathologies of common diseases such as cancer, cardiovascular diseases (CVD), diabetes, and neurodegenerative diseases. Although redox status can be probed by various methods, these methods are either invasive or not specific to NAD-coupled redox state. There is a great need of non-invasive methods to measure the tissue redox state NAD^+/NADH *in vivo*. Here we present a non-invasive hyperpolarized ^{13}C-NMR method to quantify the cytosolic NAD^+/NADH in human breast cancer mouse xenografts.

Materials and Methods

Quantification of the cytosolic NAD^+/NADH *in vivo* can be achieved by considering the lactate dehydrogenase (LDH) reaction, i.e., pyruvate+NADH+H^+ --> L-lactate+NAD^+, which is catalyzed by lactate dehydrogenase present mainly in cytosol of all tissues.[3] With the equilibrium constant K = 1.11×10^{-11} M for LDH reaction,[3] the NAD^+/NADH ratio can be obtained by $[NAD^+]_{cyto}/[NADH]_{cyto} = ([\text{pyruvate}]/[\text{lactate}])\cdot([H^+]_{cyto}/K) = (k_l/k_p)\cdot(10^{-pH_i}/K)$ (1), in which k_p and k_l are the forward and backward reaction rate constants and pH_i is the intracellular pH. The ratio k_l/k_p can be measured using hyperpolarized ^{13}C-pyruvate NMR and pH_i can be measured by ^{31}P-NMR. The athymic nude mice bearing subcutaenous xenografts of breast cancer cell line MDA-MB-231 or MCF-7 were anesthetized with isoflurane-doped oxygen flow and the body temperature was maintained ~35.0±2.5 ^{0}C. 250µl 75mM ^{13}C-1-pyruvate hyperpolarized by the DNP method (GE HyperSense) was injected into mouse xenografts via tail vein. A series of ^{13}C-NMR spectra were obtained by a single pulse sequence (TR 2s, acquisition time 1s, flip angle 9^0, bw ~20K Hz) for ~2 min using a home-built ^{1}H/^{13}C double tuned slot resonator (11mm or 14 mm in diameter) on a vertical bore 9.4-T Varian NMR spectrometer. A slice-selective gradient was used for localized signal acquisition of small tumors with low filling factors. The raw data were then processed with FFT using a customized Matlab software with 20Hz line broadening and an automatic baseline correction procedure.[5] The signals of lactate and pyruvate, $L(t)$ and $P(t)$, were obtained by fitting the raw data to Lorentian function. Furthermore, using the two-site chemical exchange model,[6] the ratio $R_{lp}(t)= L(t)/P(t)$ was fit to $R_{lp}(t) = \left\{k_p/k_l\,(1+R_{lp}(t_0)) + \left[R_{lp}(t_0) - k_p/k_l\right]e^{-(k_p+k_l)(t-t_0)}\right\} \Big/ \left\{1+R_{lp}(t_0) + \left[k_p/k_l - R_{lp}(t_0)\right]e^{-(k_p+k_l)(t-t_0)}\right\}$ (2) to obtain k_p/k_l. The localized ^{31}P-spectra of the mouse xenografts were also acquired separately using an ISIS sequence with a ^{1}H/^{31}P slot resonator (10 mm) on a 9.4-T horizontal bore Varian NMR spectrometer. The tumor pH_i was determined by plugging the chemical shift difference δ between the P_i and αNTP peaks into the Henderson-Hasselbach equation (pK_a=6.65 and chemical shift constants are 13.25ppm and 10.85ppm).[7]

Results

Fig. 1 shows the hyperpolarized ^{13}C-NMR data for a representative tumor MDA-MB-231 (#90). Fig. 1a depicts the time course of lactate and pyruvate; Fig 1b shows the ratio $R_{lp}(t)$ vs t and the fit curve for t range 20~40s (R^2=0.97). This range corresponds to the period after pyruvate reached tissue and before $P(t)$ decayed to the noisy background and $R_{lp}(t)$ started to fluctuate significantly. Fig. 2 shows a typical ^{31}P-NMR spectrum of a MDA-MB-231 tumor that was used for pH determination.

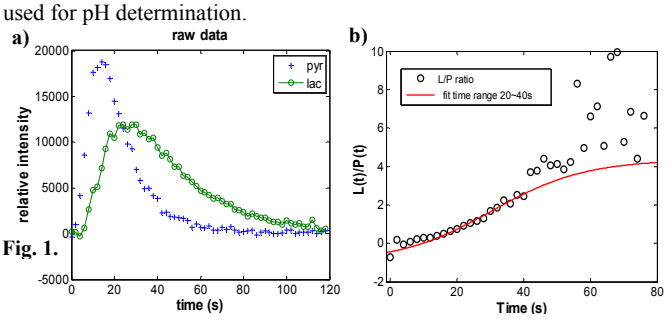


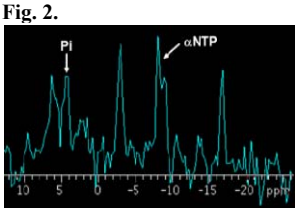
Fig. 1.

Table 1. Summary of the results from four breast tumors.

Tumor #	size (mm^3)	δ (ppm)	pH_i	k_p/ k_l	NAD^+/NADH ($\times10^3$)
90 (MDA-231)	1380	-	[a]6.88	4.4	2.7
665 (MDA-231, [b]ss)	81	12.56	6.99	1.5	6.1
674R (MCF-7, ss)	228	12.57	7.00	2.8	3.2
675L (MCF-7, ss)	140	12.60	7.03	3.4	2.5

[a] pH_i not measured for this tumor and average pH_i of MDA-231 xenografts (N=4) used; [b]Slice selective sequence used.

Fig. 2.



Discussion and Conclusion

We have shown preliminary data to quantify the cytosolic redox state NAD^+/NADH noninvasively in breast cancer mouse xenografts. The reported values of NAD^+/NADH in normal tissue such as liver varies from several hundred to a couple of thousands as measured by biochemical assays.[3] Our results are approximately on the same order of magnitude but higher. This discrepancy may be explained by possibilities including that (1) the cellular redox state of tumor tissue may be different from normal tissue; (2) it is unknown whether K *in vivo* deviates significantly from $1.11\text{x}10^{-11}$ M that was measured *in vitro*. However, the NMR measurement of the tissue redox state *in vivo* can be calibrated by the direct biochemical measurement of the tissue NAD^+/NADH in the future.

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