Measurement of Citrate Chemical Shift Changes in HR-MAS NMR Spectra of Prostate Biopsies

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INTRODUCTION: Certain metabolites visible in tissue via high resolution magic angle spinning (HR-MAS) NMR can demonstrate variations in chemical shift or coupling constant due to interactions with changing levels of other endogenous chemicals. In prostate tissue the chemical shift of citrate can change depending on the presence of of cations and pH[1,2]. This change has potential diagnostic implications, as the levels of zinc, a prominent cation in healthy prostate tissue, are known to decrease in the early stages of cancerous transformation. Thus measurable effects of zinc levels on citrate chemical shift could serve as a key marker for progression to cancer. Due to the low spectral dispersion obtainable for spectroscopy at 1.5T, chemical shift changes in citrate may be unmeasurable *in vivo*[1]; however, this study aims to demonstrate the feasibility of their detection with HR-MAS NMR at 11.74T. In this study, chemical shifts and their changes in citrate are reported and compared between healthy glandular and cancer prostate biopsy tissue.

METHODS: Transrectal ultrasound guided biopsies (N=24) were obtained from 22 patients and stored at -80°C until HR-MAS spectroscopy. HR-MAS data were acquired at 11.74 T (500 MHz for ^{1}H), 1°C, and 2,250 Hz spin rate using a Varian INOVA spectrometer, equipped with a 4mm gHX nanoprobe. 3.0 µl of D_2O containing 0.75% TSP (D_2O+TSP) was pipetted into the bottom of a 10 µl zirconium rotor and weighed, after which the tissue samples were weighed and added to the rotor. 1D "presat" spectra were acquired with 2s presaturation, 2s acquisition, 64 transients, 40,000 data points, 20,000 Hz spectral width, and a 90° flip angle. The data were zerofilled, Fourier transformed, phased, and referenced to TSP (0 ppm). The chemical shifts of the four peaks of citrate and also of creatine (as a control) were measured in an automated fashion. The J-coupling and Δ of citrate were calculated from the peak locations. Δ is a measure of the spin system and is calculated as $\Delta^2 = X^2 - J^2$, where J is the coupling constant, and X is the difference in frequency between the center of the first citrate doublet and the center of the second citrate doublet (Figure 1). The data were divided into two categories according to tissue composition following histological evaluation by a pathologist: healthy glandular (>40% glandular) and cancer (≥20% cancer). Samples included in the analysis had a mean SNR of 14.5 (σ = 7.3) on the smaller outer peaks. Healthy stromal samples and a few cancer samples were excluded from the analysis as their outer citrate peaks had a lower SNR. The peak location of creatine, and the J-coupling and Δ of citrate were compared between tissue types using a two tailed t test.

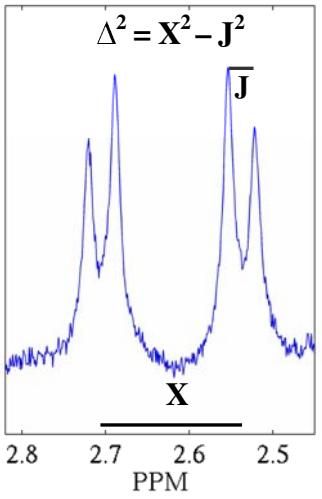

Figure 1: Measurement of Δ in the citrate region of a healthy glandular prostate spectra. X and J are indicated on the plot and both decrease to cause an overall decrease in Δ in prostate cancer.

RESULTS AND DISCUSSION: Healthy glandular tissue has high citrate levels due to a zinc-mediated inhibition of aconitase, the enzyme that converts citrate to isocitrate[1]. When the tissue progresses to cancer, decreases in zinc levels allow citrate turnover to occur. These biochemical changes in the prostate during the progression to cancer have interesting effects on the NMR properties of citrate. Citrate, a prevalent polyanionic metabolite in the prostate, resonantes as a doublet of doublets in a high magnetic field (Figure 1). However, the location of these four peaks are affected by the presence of cations. pH also can affect citrate peak location, but it has been shown that the cation effects dominate in the physiological pH range[1]. Zinc is a key cation in the normal prostate, whose levels are known to fall early in the progression to cancer. In vitro, citrate resonances have been shown to vary with zinc concentration: falling zinc levels cause Δ and J-coupling to decrease[1]. Thus, Δ and J-coupling should change in cancer tissue. We show a significant drop in Δ [Δ = 79.2 Hz (cancer) vs 81.2 Hz (glandular), P<0.05, Figure 2] as both X and J are lower in cancer, and notice a trend reduction in J-coupling between healthy glandular and cancer tissue. As falling zinc precedes falling citrate in the progression to cancer, this change in Δ could serve as an earlier marker for progression to cancer.

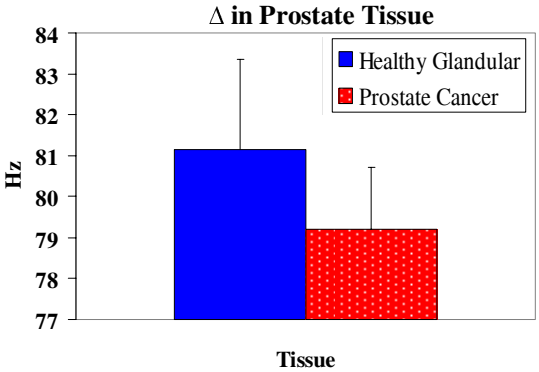

Figure 2: Δ decreases in prostate cancer, due to changes in the microenvironment of citrate.

CONCLUSIONS: The results demonstrate that Δ drops significantly between healthy glandular and prostate cancer tissue. The trend in J-coupling changes also agrees with the cation effect on citrate. Since Δ can also be affected by pH and other cations, direct measurements of zinc by atomic absorption spectroscopy in the same tissues are necessary to establish a relationship between zinc concentration and changes in Δ.

REFERENCES:

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