

Investigation of choline and ethanolamine containing compounds in human prostate tissues by 2D HR-MAS TOCSY

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Introduction

Previous ¹H and ³¹P MRS studies on tissues and extracts have identified increased choline and ethanolamine containing compounds in malignant vs. benign prostate tissues (1,2). In vivo and ex vivo ¹H MRS studies have focused largely on the so-called "choline region" from 3.20 to 3.24 ppm where the choline head group resonates with 9 equivalent protons. However, within this region, the individual compounds free choline (Cho), phosphocholine (PC), and glycerophosphocholine (GPC) cannot be completely resolved and there is extensive overlap with ethanolamine (Eth), phosphoethanolamine (PE), glycerophosphoethanolamine (GPE), myo-inositol, taurine (Tau), and polyamines. It has recently been shown (3) that the side chain CH₂-CH₂ crosspeaks of choline and ethanolamine containing compounds can be completely resolved and quantified using 2D Total Correlation Spectroscopy (TOCSY). However, in order to minimize metabolic and pathologic degradation in intact tissues, the data must be acquired as quickly as possible. In this study, a protocol was developed to resolve and quantify individual choline and ethanolamine containing compounds in healthy and malignant prostate tissues in 1 hour using rotor synchronized adiabatic TOCSY experiments.

Methods

Data were acquired at 11.5T (500 MHz), 1°C, and a 2,250 Hz spin rate using a Varian INOVA spectrometer equipped with a 4 mm gHX nanoprobe. Twenty patient-matched post-surgical prostate tissues (n = 10 healthy glandular, n = 10 cancer) were studied using custom designed 18 µl rotors. 3.0 µl D₂O+0.75% TSP (Aldrich) was added to each rotor and weighed, after which the tissue sample was weighed (mean = 13.2 ± 2.4 mg) and added. 1D presat spectra (TR = 6s, AT = 2.2s, NT = 32, 3:36 min/ea) were acquired before and after each 2D experiment. TOCSY data were acquired using a rotor synchronized adiabatic (WURST-2) mixing scheme (4) with 1s presat-relaxation delay (TR=1.24s), AT = 0.2s, τ_m = 40 ms, NT = 24, SW = 20,000 × 6,000 Hz, NP= 4096 × 64 complex points, time = 1.08 hrs. The total time for sample prep, shimming, and data acquisition was about 1.5 hours. Serial 1-hour TOCSYs were also performed on 3 samples for 9 hours to study the degradation of choline and ethanolamine containing compounds over time. Following HR-MAS, samples were submitted in formalin for pathologic (H&E) analysis. Data were processed using 3×N linear prediction in F1, zero-filled to 4K (F2) and 2K (F1) complex points, and apodized using a Gaussian function in both dimensions. Crosspeaks were volume integrated and total choline and ethanolamine volumes were normalized to the Tau diagonal peak at 3.43 ppm.

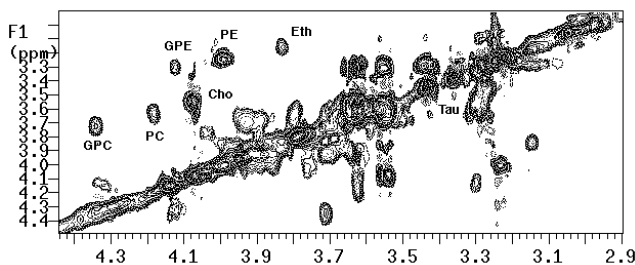


Figure 1. 40 ms TOCSY spectrum of prostate cancer tissue (Gleason 3+3, 12.7 mg) showing resolution of choline and ethanolamine containing compounds.

Results

TOCSY acquisition parameters were first optimized to ensure that adequate resolution and signal to noise (S/N) could be obtained routinely in one hour. Signal to noise rather than resolution was found to be the most critical factor for successful studies, with NT=8 to 16 transients/increment producing marginal S/N, and NT=24 transients/increment providing sufficient S/N provided the sample size was at least 10 mg. Initial studies also determined an optimal mixing time of 40 ms for the 3 bond CH₂-CH₂ couplings found in choline and ethanolamine compounds and that 64 increments (complex) was sufficient to resolve each crosspeak in the indirect dimension. Figure 1 shows a portion of a 40 ms adiabatic TOCSY spectrum of prostate cancer tissue showing the six crosspeaks of GPC, PC, Cho, GPE, PE, and Eth, and the Tau diagonal peak.

PC/GPC ratios were significantly higher in prostate cancer (mean=0.94) vs. healthy glandular (mean=0.17) tissues (p=0.006). PE/GPE was higher (but not significantly) in prostate cancer (mean = 5.10) vs. healthy glandular (mean = 4.02) tissues; however, PE/Eth was significantly higher in prostate cancer (mean=5.27) vs. healthy glandular (mean=2.02) tissues (p=0.042). Total choline and ethanolamine to Tau ratios were both significantly higher in prostate cancer (mean=0.68, 0.74) vs. healthy glandular (mean=0.50, 0.54) tissues (p=0.0497, 0.018). Degradation studies showed a 6.0% and 5.2% decrease in GPC and PC per hour, respectively, while Cho increased by 3.7% per hour. GPE and PE each decreased by 2.2% per hour, while Eth increased by 7.0% per hour. The change in the Tau diagonal peak volume was negligible over time.

Discussion

Spectral overlap is a major problem in ¹H HR-MAS studies of intact tissues, even at 11.5 T (500 MHz). By applying techniques which spread the data into two dimensions, the overlapping peaks can often be completely resolved. TOCSY data can be quantified because they produce absorption phase lineshapes and the efficiency of coherence transfer is more or less independent of the scalar coupling constants of the individual components. Because the same type of crosspeak (i.e., CH₂-CH₂) was quantified for each metabolite, the relaxation times of the individual protons were assumed to be similar and hence partial saturation was not considered further. Degradation studies showed less than 10% change per hour for each metabolite investigated, indicating that the metabolite levels remained fairly stable over the duration of each experiment.

Conclusions

This study demonstrated that individual choline and ethanolamine containing compounds could be completely resolved and quantified in a reasonable amount of time using 2D HR-MAS TOCSY experiments, and that this technique could be used to detect significant differences in choline and ethanolamine containing compounds between healthy and malignant prostate tissues.

References

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