Single-Voxel Oversampled J-Resolved Spectroscopy of In Vivo Human Prostate Tissue

Mark G. Swanson,1 Daniel B. Vigneron,1 Tuan-Khanh C. Tran,1 Napapon Sailasuta,2 Ralph E. Hurd,2 and John Kurhanewicz1*

Single-voxel J-resolved spectroscopy with oversampling in the F1 dimension was used to obtain water unsuppressed 1H spectra of in situ human prostate tissue in 40 previously untreated prostate cancer patients. Based on T2-weighted MRI and previous biopsy information, voxels were placed in regions of benign or malignant peripheral zone tissue, or in regions of predominantly glandular or stromal benign prostatic hyperplasia (BPH) within the central gland. The addition of a second J-resolved dimension allowed for the observation of the J-modulation of citrate, as well as the resolution of polyamines from overlapping choline and creatine signals. Regions of healthy peripheral zone tissue and glandular BPH all demonstrated high levels of citrate and polyamines, with consistent coupling and J-modulation patterns. Conversely, regions of malignant peripheral zone tissue and stromal BPH demonstrated low levels of citrate and polyamines consistent with prior in vivo and ex vivo studies. Moreover, water T2 relaxation times determined for healthy peripheral zone tissue (mean 128 ± 15.2 msec) were significantly different than for malignant peripheral zone tissue (mean 88.0 ± 14.2 msec, P = 0.005), as well as for predominantly glandular (mean 92.4 ± 12.2 msec, P = 0.009) and stromal BPH (mean 70.9 ± 12.1 msec, P = 0.003). This preliminary study demonstrates that J-resolved spectroscopy of the in situ prostate can be acquired, and the information obtained from the second spectral dimension can provide additional physiologic information from human prostate tissue in a reasonable amount of time (< 10 min). Magn Reson Med 45:973–980, 2001. © 2001 Wiley-Liss, Inc.

Key words: J-resolved spectroscopy; spectroscopic imaging; prostate cancer; citrate; polyamines

Previous 3D magnetic resonance spectroscopic imaging (3D-MRSI) studies have distinguished prostate cancer from healthy peripheral zone (PZ) tissue with high specificity (1). This high specificity is due to changes in both citrate and choline metabolism which result in increased (choline + creatine)/citrate ratios in the presence of cancer (2). However, in many cases, prostate cancer is still difficult to detect, because these metabolic changes may be too small for a conclusive or “clear-cut” diagnosis. This situation is common in infiltrative tumors or early-stage disease, where there is significant partial voluming with surrounding healthy tissues. Therefore, additional metabolic or physiologic markers that can discriminate prostate cancer at earlier stages of progression are needed.

Three-dimensional MRSI spectra of the human prostate at 1.5 T typically contain significant spectral overlap, particularly in the choline region. This spectral overlap could potentially be resolved using various 2D NMR techniques that have been adapted for clinical MRI scanners (3–5). Localized single-voxel 2D J-resolved spectra of the human brain have been obtained using a point-resolved spectroscopy (PRESS) (6) localization scheme, modified with an incremented evolution time surrounding the final slice-selective 180° RF pulse (4,5). While these early studies invariably relied on water suppression (e.g., chemical shift selected (CHESS) (7)), Hurd et al. (8) recently showed that 2D J-resolved spectra could be obtained in the human brain without presaturation by oversampling in the F1 dimension. In this method, the echo time (TE) was incremented to not only sample the narrow F1 bandwidth required to cover the range of metabolite proton-proton couplings, but also to resolve the coherent gradient-induced water sidebands. These sidebands have historically been one of the primary sources of water-induced baseline problems in vivo proton spectroscopy, and their elimination allowed for the acquisition of water-unsuppressed localized proton spectra within the brain. A full water signal is ideal as a quantitation, phase, and frequency reference, and is essential for obtaining water relaxation information. Additionally, the second “J” dimension resolves homonuclear coupled resonances from overlapping singlets such as choline and creatine. The purpose of this study was to determine the feasibility of acquiring 2D J-resolved spectra from the in situ prostate, and what additional biochemical information this technique could provide.

MATERIALS AND METHODS

Forty patients (mean age = 60 ± 10 years) were studied as an addition to an MRI/3D-MRSI staging exam for prostate cancer (2). All patients were previously untreated and had biopsy-proven prostate cancer (mean Gleason sum = 5.9 ± 0.8), except for four who had rising prostate specific antigen levels, but had either no prior biopsy (N = 1) or had prior negative biopsies (N = 3). All studies were performed using a 1.5T GE MR Sigra scanner (GE Medical Systems, Milwaukee, WI). The body coil was used for signal excitation and an endorectal/pelvic phased array coil system (Medrad, Pittsburgh, PA) was used for signal reception. The protocol for the acquisition of MRI and 3D-MRSI data has previously been described (9). MRI images were analytically corrected for the inhomogeneous reception profile of the endorectal and pelvic phased array coils (10).

1Magnetic Resonance Science Center, Department of Radiology, University of California–San Francisco, San Francisco, California.
2GE Medical Systems, Fremont, California.
Grant sponsor: American Cancer Society; Grant number: RPG-96-146-03-CCE. Grant sponsor: National Institutes of Health; Grant numbers: RO1 CA79886; R29 CA64867; RO1 CA59897; Grant sponsor: CapCURE Foundation.
*Correspondence to: John Kurhanewicz, Ph.D., Magnetic Resonance Science Center, Department of Radiology, 1 Irving Street, Room AG-109, University of California–San Francisco, San Francisco, CA 94143-1290. E-mail: johnk@mrsc.ucsf.edu
Received 26 April 2000; revised 21 November 2000; accepted 18 December 2000.

© 2001 Wiley-Liss, Inc.
Based on biopsy data and contiguous $T_2$-weighted MR images acquired during the same study (TR/TE = 6000/108 msec effective, 3-mm sections/interleaved, 3 signals acquired, 14-cm field of view, 256 × 192 matrix, no phase wrap), voxels of interest were selected to be in regions of healthy ($N = 11$) or malignant ($N = 17$) peripheral zone tissue or in regions of predominantly glandular or stromal benign prostatic hyperplasia (BPH) within the central gland ($N = 12$). For the four patients with negative or no prior biopsies, voxels were chosen based on imaging and then categorized as healthy or malignant based upon combined imaging and spectroscopy findings. Voxels were prescribed as carefully as possible to avoid the air-tissue interface of the rectum, periprostatic lipids, and mixed tissues. Typical voxel dimensions were $12 \times 12 \times 15$ mm ($\pm 2.16$ cm$^3$) in the R/L, A/P, and S/I dimensions, respectively. The mean voxel sizes were $1.9 \pm 0.6$ cm$^3$ in regions of healthy peripheral zone tissue, $1.9 \pm 0.8$ cm$^3$ in regions of malignant peripheral zone tissue, and $9.8 \pm 7.2$ cm$^3$ in regions of glandular or stromal BPH.

Single-voxel J-resolved spectra were acquired with a modified asymmetric PRESS localization scheme given by:

$$90 - \text{TE}_1 - 180 - \text{TE}_2 - \tau_1/2 - 180 - \tau_2/2 - \tau_1 (\text{acquire}),$$

where the initial echo time, $\text{TE} = \text{TE}_1 + \text{TE}_2$, was 35 msec, and $\tau_1$ was the incremented evolution time. Experimental optimization and validation were performed using phantom solutions (pH $\pm 7$) containing either 10 mM choline, 15 mM creatine, 90 mM citrate, and 35 mM lactate, or 20 mM spermine. The first 10 patients were then studied using various acquisition parameters (3 or 4 Hz per point spectral resolution, two or four excitations, 64–128 $\tau_1$ increments, 1.4- or 2.0-sec TR) in order to optimize the parameters for in vivo conditions. For the remaining 30 patients, the following set of parameters was used: 512 data points were acquired over a 1000 Hz spectral width in the $\tau_2$ dimension with the transmitter placed on water. In the $\tau_1$ dimension, 64 increments were acquired over a 256 Hz spectral width to yield an $F_1$ resolution of 4 Hz per point. This resolution was chosen based upon the coupling constant of citrate ($\pm 16$ Hz), whereas, in the previous report, the $F_1$ spectral resolution (3.13 Hz per point) was based upon the coupling constant of lactate ($\pm 7$ Hz) (8). Finally, four excitations were acquired per increment with a repetition rate of 2.0 sec, such that the total acquisition time was 8:32 min.

J-resolved data were processed and displayed using SAGE (General Electric, Milwaukee) for IDL (Research Systems, Inc., Boulder, CO) software on a Sun Ultra 10 workstation (Sun Microsystems, Palo Alto, CA). Two-dimensional water referencing was used for initial phase and frequency corrections. The raw data were then apodized using a 10–12% shifted 2–3 Hz Gaussian function in the $F_2$ dimension prior to Fourier transformation. No apodization was employed in the $F_1$ dimension. Two-dimensional spectra were baseline corrected using a cubic spline method, which was optimized using the center $J(0)$ line of the spectra and then applied to the rest of the data. For determination of water $T_2$ relaxation times, data were Fourier transformed in the $F_2$ dimension only, and then fitted to standard single and double exponential equations. $T_2$ relaxation times were statistically compared using a two-tailed Student’s $t$-test. Because of the complex J-modulation of the citrate AB spin system, no attempt was made here to calculate $T_2$ relaxation times for citrate.

RESULTS

Figure 1 illustrates the effect of $\tau_1$ oversampling on a phantom spectrum acquired under the same conditions as the majority of patient studies. Consistent with the previous report in the brain (8), 64 $\tau_1$ increments adequately resolved coherent gradient artifacts from the signals of interest within the prostate. Figure 2a shows the $\pm 16$ Hz region of the oversampled 2D J-resolved phantom spectrum shown in Fig. 1. For clarity, each slice except the center $J(0)$ slice has been scaled up by a factor of 2. Because choline and creatine are singlets, arising from nine and three equivalent protons, respectively, no proton couplings are expected along either the $J(4)$ or $J(8)$ Hz lines. The coupled spins of citrate, however, give rise to cross signals at $J(\pm 4)$, $J(\pm 8)$, and $J(\pm 16)$ Hz, and lactate gives rise to signals at $J(\pm 4)$ Hz. Figure 2b shows an oversampled 2D J-resolved spectrum of a phantom solution containing spermine. Spermine resonances are observed along $J(0)$ at 1.8 ppm, 2.1 ppm, and 3.15 ppm, and 2D crosspeaks for spermine are observed along each of the $J(\pm 4)$, $J(\pm 8)$, $J(\pm 12)$, and $J(\pm 16)$ Hz lines. Figure 2c demonstrates the J-modulation that would be observed in vivo if the major contributions to the in vivo spectrum were from choline, creatine, citrate, lactate, and polyamines.

Figure 3 shows the $\pm 8$ Hz region of the complete 2D J-resolved spectrum for a representative region of healthy
peripheral zone tissue and, for comparison, MRI/3D-MRSI data from the same patient exam. The T2-weighted MRI image (Fig. 3a) and 3D-MRSI data shown (Fig. 3b) correspond to the slices at the center of the J-resolved spectroscopy voxel (Fig. 3c) in the superior/inferior (S/I) dimension. The corresponding J(0), J(+4), and J(+8) Hz lines are shown in Fig. 3d and the metabolites choline (Cho), creatine (Cr), polyamines (PA), and citrate are identified. The 3D-MRSI pattern shown in Fig. 3b is typical of healthy peripheral zone tissue and demonstrates low levels of choline and high levels of citrate (2). Along the center J(0) line of the J-resolved spectrum (Fig. 3d), low levels of choline and high levels of citrate are also observed. The complex J-modulation of the citrate multiplet appears very similar to that shown in Fig. 2a, with the inner lines of the doublet of doublets being large and positive along the J(+4) Hz lines and the outer lines of the multiplet being smaller and inverted along the J(+8) Hz lines. Polyamines (PA) (spermine, spermidine, and putrescine) are buried underneath choline, creatine, and lipids in the 1D 1H spectrum at 1.5T; however, their J-modulation can be clearly observed along the J(+4) and J(+8) Hz lines in Fig. 3d, similar to the phantom results shown in Fig. 2b. Ten of the 11 regions of interest (ROIs) targeted as healthy tissue were acquired under the same set of conditions. However, in only four cases was the signal-to-noise ratio (SNR) high enough to quantitate the J-modulation of metabolites. In each of these four cases, polyamine and citrate signals were very strong along the J(0), J(+4), and J(+8) Hz lines, and the J-modulation of polyamines and citrate appeared identical to that shown in Figs. 2 and 3.

Figure 4 shows a 2D J-resolved spectrum obtained from a large region of prostate cancer and the corresponding MRI/3D-MRSI data from the same patient exam. In the presence of prostate cancer, the amount to which choline is elevated and citrate is reduced correlates with the overall aggressiveness of the tumor (11). The MRI/3D-MRSI findings shown in Fig. 4a and b are consistent with an aggressive large-volume tumor encompassing the entire right side of the gland. The J-resolved spectrum shown in Fig. 4d demonstrates elevated choline, which appears to be resolved from creatine along the J(0) Hz line because there is less spectral overlap in this region due to an absence of polyamines. In the cancer, citrate was nearly undetectable, with weak signals appearing along the J(0) and J(−4) Hz lines. Similarly, polyamines are nearly absent along the J(+4) Hz lines, confirming previous ex vivo reports that polyamines are reduced in the presence of prostate cancer (12,13). Nine of the 17 ROIs targeted as malignant peripheral zone tissue were acquired under the same set of conditions. However, in only six of these cases were the prostatic metabolites choline, creatine, and citrate present with sufficient SNR to be quantitatively useful. In each of these cases, polyamines were either dramatically reduced or absent altogether, and citrate was reduced in each case as well. In four of these cases, choline levels were noticeably elevated relative to healthy spectra.

Benign prostatic hyperplastic (BPH) tissue is comprised of glandular and stromal components, and in many cases may be predominantly composed of one or the other. Glandular BPH appears very similar to healthy peripheral zone tissue due the presence of high levels of citrate and high signal intensity on T2-weighted MRI, whereas stromal BPH may resemble cancer due to a reduction or absence of citrate and lower signal intensity on T2-weighted MRI. Figure 5 shows a J-resolved spectrum and MRI/3D-MRSI data obtained from a region of predominantly glandular

![Image](image-url)
BPH within the central gland. In the MRSI array (Fig. 5b) example voxels containing primarily glandular (G) (evidenced by the large duct in the center of Fig. 5a) and stromal (S) tissue are identified. The single-voxel J-resolved spectrum covering the region shown in Fig. 5c demonstrates low choline and creatine levels and very high citrate levels (Fig. 5d). As in Fig. 3, citrate and polyamines can be observed along the J(±4) and J(±8) Hz lines.

The observation of polyamine signals in the J(±4) and J(±8) Hz lines confirms their presence in regions of glandular BPH.

Eleven of the 12 ROIs targeted as BPH were acquired under the same set of conditions. In 10 of these 11 cases, the prostate metabolites choline, creatine, citrate, and polyamines were present with great enough SNR to be quantitatively useful. The large number of useable studies here reflects the larger voxel sizes that can be placed in the central gland versus regions of healthy or malignant peripheral zone tissue. Of these 10 cases, seven were in regions of predominantly glandular BPH, and three were in regions of predominantly stromal BPH. In each of the predominantly glandular BPH studies, polyamine and citrate levels were very high, and their J-modulation patterns were indistinguishable from that shown in Figs. 2, 3, and 5. In each of the predominantly stromal BPH studies, citrate levels were reduced, and polyamines were undetectable, consistent with the absence of glandular components in predominantly stromal tissue.

Transverse Relaxation Times

For the 20 patients who had quantitatively useable spectra, water $T_2$ relaxation times were determined for regions of benign and malignant peripheral zone tissue, and predominantly glandular and stromal BPH. $T_2$ relaxation times for healthy peripheral zone tissue ($N = 4$) ranged from 113 to 147 msec (mean 128 ± 15.2 msec) while $T_2$ relaxation times for malignant peripheral zone ($N = 6$) ranged from 65.7 to 103.0 msec (mean 88.0 ± 14.2 msec). $T_2$ relaxation times for predominantly glandular BPH ($N = 7$) ranged from 68.5 to 102 msec (mean 92.4 ± 12.2 msec), and for stromal BPH ($N = 3$) ranged from 59.6 to 84.8 msec (mean 70.9 ± 12.1 msec). Mean water $T_2$ relaxation times for healthy peripheral zone tissue were significantly different from malignant peripheral zone tissue ($P = 0.005$) as well as predominantly glandular (0.009) and stromal BPH ($P = 0.003$). In addition, water $T_2$ relaxation times for glandular BPH were longer than for stromal BPH, but the difference was not significant ($P = 0.12$), presumably due to the small number of stromal BPH studies. Additionally, there was some overlap of individual water $T_2$ relaxation times in malignant tissue with benign peripheral zone tissues, and extensive overlap with predominantly stromal BPH such as...
that the differences in the latter case were not significant ($P = 0.56$).

**DISCUSSION**

Much of the success of previous 3D-MRSI studies of the prostate is due to multiple changes in citrate and choline metabolism that occur during pathogenesis. The specificity of cancer detection could be further increased by combining additional metabolic markers, monitoring changes in citrate J-modulation patterns, and incorporating water and metabolite relaxation information. This study demonstrates the capability of oversampled 2D J-resolved spectroscopy to obtain water-unsuppressed spectra from the in situ prostate and provide additional metabolic information that cannot be obtained from conventional 1D MR spectra. The presence of a full water signal is necessary for determining water relaxation times, and also provides a phase and frequency reference. Figure 1 demonstrates that oversampling in $T_1$ spreads spurious sidebands of water away from the signals of interest in the prostate. The phantom and in vivo results presented in Figs. 2–5 illustrate some of the potential biological information that can be obtained using this technique.

The addition of a second J-resolved dimension allowed for the observation of the J-modulation of citrate, as well as the resolution of polyamines from overlapping choline and creatine signals. Regions of healthy peripheral zone tissue and glandular BPH all demonstrated high levels of citrate and polyamines, with consistent coupling and J-modulation patterns. Because the methylene protons of citrate comprise a strongly coupled AB spin system, the signal intensities and phase distortions of the four lines of the multiplet depend not only on the coupling constant (J) and chemical shift difference ($\Delta$) but also the type and timings of the pulse sequence used (14–18). If the same pulse sequence is used, changes in J-modulation may be correlated with pathology and used as an additional marker for prostate cancer.

The J-modulation of citrate could be altered by changes in zinc levels in the prostate. The unique capability of healthy prostate epithelial cells to synthesize, store, and secrete unusually large amounts of citrate (19,20) results directly from the presence of high concentrations of zinc in the prostate, some of which is bound to citrate. Zinc inhibits the enzyme aconitase, which in turn prevents the oxidation of citrate via the Krebs cycle. However, in the presence of prostate cancer, zinc concentrations drop sharply, citrate turnover increases, and prostate epithelial cells lose their ability to accumulate citrate (21,22). While zinc is not NMR active, J-resolved spectroscopy may provide a means for indirectly detecting zinc changes in the prostate by detecting changes in citrate J-modulation, thereby providing an additional early marker for prostate cancer.

**FIG. 4.** Oversampled 2D J-resolved spectrum from a 3.7 cm$^3$ region of prostate cancer: (a) axial $T_2$-weighted image, (b) bilateral 3D-MRSI spectral array, (c) axial $T_2$-weighted image showing 2D J-voxel location, and (d) 2D J-resolved spectrum.
cancer (23). In the present study, low levels of citrate were observed in regions of prostate cancer and predominantly stromal BPH. However, because of the lower SNR of the outer citrate lines in regions of prostate cancer, we were not able to detect significant differences in J-modulation of citrate between healthy and malignant tissues in this study.

This study also demonstrated the ability of 2D J-resolved spectroscopy to resolve another possible metabolic marker, polyamines, from overlapping choline and creatine singlets. In the present study, high levels of polyamines were observed along the J(0), J(±4), and J(±8) Hz lines in regions of healthy peripheral zone tissue and predominantly glandular BPH, whereas in regions of prostate cancer, polyamines were nearly or completely absent. The polyamines spermine, spermidine, and putrescine are essential for the differentiation and proliferation of cells, the synthesis of DNA, RNA, and proteins, and the stabilization of cell membranes and cytoskeletal structures (24). Van der Graaf et al. (12) observed high levels of spermine in healthy prostate tissue and BPH and reduced spermine levels in malignant prostate tissue. Preliminary evidence from 1H high-resolution magic angle spinning (1H HR-MAS) NMR experiments of postsurgical prostate tissue also showed that polyamines are elevated in healthy glandular tissue and are reduced or absent in the presence of prostate cancer (13). The results of these and the present study suggest that a reduction in polyamines could be useful as an early marker for prostate cancer.

The elevation of in vivo “choline” levels is a common feature of prostate cancer, particularly higher grade tumors. Unlike the in vivo citrate resonance, which is well resolved at 1.5 T, the in vivo “choline” resonance contains contributions from several compounds, including choline (Cho), phosphocholine (PC), glycerophosphocholine (GPC), polyamines (PA), ethanolamine, phosphoethanolamine (PE), inositol, and taurine. While choline-containing compounds give rise to singlets which are only observed along the J(0) line, coupled protons from ethanolamines, inositol, taurine, and particularly polyamines can each contribute to the signals observed along the J(±4) and J(±8) Hz lines of the J-resolved spectrum. In addition to polyamines being reduced or absent in regions of prostate cancer.

**FIG. 5.** Oversampled 2D J-resolved spectrum from a 17.1 cm³ region of predominantly glandular BPH: (a) axial T₂-weighted image, (b) corresponding 3D-MRSI spectral array, (c) axial T₂-weighted image showing 2D J-voxel location, and (d) 2D J-resolved spectrum.
Oversampled J-Resolved Spectroscopy

Single-voxel water-unsuppressed J-resolved spectra in vitro in BPH. Water T₂ relaxation times determined in this study were in agreement with previously published results in healthy volunteers (25,26). BPH, and prostate cancer patients (27). In regions of prostate cancer, we observed a significant reduction of water T₂ relaxation times as compared to healthy peripheral zone tissues. However, there was some overlap of individual water T₂ times between cancer and healthy peripheral zone tissue, and extensive overlap between cancer and predominantly stromal BPH. T₂ relaxation times for choline determined in this preliminary study were not reliable due to insufficient SNR, particularly in healthy tissues, where choline levels are normally low and spectroscopy voxels are relatively small.

SNR could be improved in future studies by using more sensitive surfaces coils, going to higher magnetic fields (3 T), acquiring data for longer periods of time, or by increasing voxel sizes. The latter option is not ideal since regions of abnormality within the prostate are often very small. A better alternative would be to employ 1D phase-encoding or spiral-based k-space sampling techniques to simultaneously provide improved spatial coverage and improved SNR (due to the longer acquisition time needed). Adalsteinsson and Spielman (28), for example, recently used spiral-based readout gradients to obtain 18 × 18 pixel spatially resolved 2D J-resolved spectra in vitro in ~17 min.

CONCLUSIONS

Single-voxel water-unsuppressed J-resolved spectra can be obtained from the human prostate in a clinically reasonable amount of time (<10 min). In this study, water-unsuppressed J-resolved spectroscopy was used to resolve polyamines from overlapping choline and creatine signals, observe the 2nd-order J-modulation of citrate, and provide T₂ relaxation information, all in a single experiment. This represents a distinct improvement compared to the quantity of information obtainable from 1D single-voxel MRS studies, and could potentially be used to further improve the specificity of prostate cancer detection by MRI/MRSI. The greatest limitation of this method is that small ROIs (~2 cm³) generally do not provide sufficient SNR to be quantitatively useful. Therefore, either longer acquisition times and/or larger voxel sizes are necessary for single-voxel acquisitions, or a phase-encoding technique with a subsequently longer acquisition time must be employed in order for the full potential of this technique to be determined.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Penelope J. Wood, Niles Bruce, Evelyn Proctor, and Pauline Bartholomew for their assistance in the acquisition and processing of the 3D MRI/MRSI and J-resolved spectroscopy data presented in this article.

REFERENCES


