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CHARACTERIZATION OF HUMAN PROSTATE CANCER, BENIGN PROSTATIC HYPERPLASIA AND NORMAL PROSTATE BY IN VITRO ^{1}H AND ^{31}P MAGNETIC RESONANCE SPECTROSCOPY

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ABSTRACT

In vitro ^{1}H and ^{31}P magnetic resonance spectra were acquired from perchloric acid extracts of human prostate tissue obtained by transurethral resection. This included tissue of patients with benign prostatic hyperplasia and prostatic adenocarcinoma; one tissue sample was obtained from a patient without any sign of BPH or malignancy.

Major resonances in the magnetic resonance spectra were assigned to prostate compounds and were quantified. The citrate/lactate, citrate/total choline, phosphocholine/total creatinine, choline/total creatine, alanine/total creatine, phosphoethanolamine/total phosphate, phosphocholine/total phosphate and glycerophosphoethanolamine/total phosphate ratios were statistically different for the prostate cancer samples as compared with the BPH specimens. These observations may contribute to the understanding of in vivo magnetic resonance spectra of the prostate and indicate that magnetic resonance spectroscopy can aid in the diagnosis of prostate malignancy.

KEY WORDS: metabolism, nuclear magnetic resonance, prostatic neoplasms, prostate, prostatic hypertrophy

Prostate cancer is a growing medical problem: it is now the most common cancer in the western male population and the second leading cause of cancer-related death in men.[1] The approach to treatment depends on the stage of the cancer at the time of diagnosis. Unfortunately, present methods for the detection of prostate malignancy (such as prostate specific antigen, transrectal ultrasonography and digital rectal examination) are inadequate to assign the precise tumor stage and to predict tumor behavior.[2] Therefore, new methods to improve tumor staging are necessary.

As yet, the clinical introduction of magnetic resonance imaging (MRI) did not significantly improve the detection and staging of prostate cancer.[3,4] Recently transrectal probes were introduced that not only improved the quality of MRI but also made the prostate accessible to magnetic resonance spectroscopy (MRS).[5-7] Magnetic resonance spectroscopy, basically employing the same instrument set-up as for MRI, is able to monitor tissue metabolism noninvasively.

^{31}P, ^{1}H and ^{13}C MRS have been used in studies that tried, both in vitro and in vivo, to associate relative levels of metabolites with benign or malignant prostate lesions.[6-14] It has been suggested that the relative levels of the phosphorylated metabolites phosphocreatine (PCr) and phosphomonoesters (PME) can be used to discriminate malignant from benign tissue.[7] Other investigators were able to measure the citrate concentration in prostatic tissue using ^{1}H MRS.[9-13] Moreover, with the help of ^{13}C MRS Sillerud and associates demonstrated a difference in relative levels of citrate between the normal and malignant prostate both in vitro and in vivo.[8,14]

The current study was designed to characterize both ^{1}H and ^{31}P MRS spectra of benign and malignant prostate at high field strengths in order to substantiate these observations and to identify further differences that may serve as markers for malignancy. Considering the spectral overlap occurring for spectra of whole tissue species, we decided to analyze perchloric acid (PCA) extracts in which resonances are relatively well resolved and easy to assign.

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MATERIALS AND METHODS

Tissues. Prostatic tissue obtained by transurethral resection (TURP) was snap frozen in liquid nitrogen within 30 seconds after the start of resection. This short ischemia time should prevent the gross occurrence of anaerobic glycolysis and breakdown of high energy phosphates. Samples ranged in size from 0.9 to 3.6 gms. Tissue of patients with benign prostatic hyperplasia (BPH) (N = 7), patients with advanced adenocarcinoma of the prostate (N = 4) and one patient with no signs of malignancy or BPH (NP) were included in this study.

Histology. Before processing the tissue samples for PCA extraction, 4 to 5 representative biopsies of one lesion (total material taken ± 0.20 gm.) were taken for histological examination to confirm the diagnosis and to correlate possible differences in the proton and phosphorus spectra with the percentage of normal, BPH, or cancerous tissue. Only for one prostate adenocarcinoma sample was it impossible to perform such an additional histological examination.

Preparation of PCA tissue extracts. Perchloric acid extraction was performed as described earlier by Smits et al.[15] Frozen tissue was weighed and pulverized at −80C and transferred into an all glass homogenizer, and 0.9 M. PCA was added dropwise to a total of five times the weight of the tumor. Complete tissue homogenization was achieved in approximately 30 minutes.

After centrifugation (12,000 g, 15 minutes, 4C) of the tissue homogenates, the pellet was discarded, and the pH of the supernatant was immediately adjusted to 7.5 with 9 M. KOH. The PCA precipitate was centrifuged (12,000 g, 15 minutes, 4C), and the supernatant was passed through a Chelex sample preparation disc (Bio-Rad Laboratories, Richmond, California) and lyophilized. Lyophilizates were stored at −20C. Thirty percent of the lyophilizate was used for ^{1}H MRS and 70% for ^{31}P MRS. Before the ^{1}H MRS measurements, the lyophilizates were carefully thawed and dissolved in 500 μl. 20 mM. potassium phosphate buffer, pH 7.0. If necessary the pH was adjusted to pH 6.9 to 7.1. Thereafter the samples were lyophilized and

dissolved in 500 μl. D_2O with 1.6 mM. 3-(Trimethylsilyl) propionic acid-d4 sodium salt (TSP).

For the ^{31}P MRS measurements the lyophilizate was dissolved in 450 μl. 0.5 mM. dimethylphosphate, 20 mM. EDTA, 10 mM. Tris, pH 7.8 in 30% D_2O.

^{31}P MRS measurements. The ^{31}P MRS spectra were acquired on a 200 mHz spectrometer (Bruker WM200) and were recorded with a standard 5 mm. ^{31}P MRS high-resolution probe employing a 50° flip angle and a pulse repetition time of 4 seconds. WALTZ-16 low power broadband ^1H decoupling was used during data acquisition. The PCA extracts were analyzed under spinning conditions with a linewidth of the H_2O signal between 1 and 3 Hz. The chemical shifts were referenced with respect to the chemical shift position of the phosphocreatine resonance.

The MRS data were further evaluated employing the NMR1 package (New Methods Research, Inc., Last Syracuse, New York) on a SUN Sparc station 330 (Sun Microsystems, Inc., Mountain View, California). FIDs were Fourier transformed, and the phase was manually corrected. The ^{31}P MR spectra were semiautomatically fitted to Lorentzian lineshape model functions. To permit comparison of different spectra, relative integrals of phosphor metabolite resonances of interest were expressed as ratio to the total integral values of all phosphate signals (TP) in the sample.

^1H MRS measurements. One and two dimensional ^1H MRS spectra were acquired on a 500 mHz spectrometer (Bruker AM500) and were recorded with a standard 5 mm. ^1H MRS probe. One dimensional ^1H MRS spectra were recorded employing a 45° flip angle and a 7.3 second pulse repetition time. The resonance of H_2O was suppressed by low power continuous wave presaturation.

The chemical shifts were referenced with respect to the chemical shift position of the TSP resonance.

^1H MRS spectra were evaluated with NMR1 software. FIDs were Fourier transformed after zero-filling from 8 K to 32 K and Lorentzian to Gaussian transformation. The phase was manually corrected and the spectra were semiautomatically fitted to Gaussian lineshape model functions. To permit comparison of different spectra, relative integrals of proton metabolite resonances of interest were expressed as ratio to the total amount of the relative integral of the methyl resonance of lactate or total creatinine (phosphocreatine plus creatine).

Homonuclear ^1H-^1H MRS measurements. For ^1H spectral assignments double-quantum filtered correlated spectroscopy (DQF-COSY) was performed using time proportional-phase incrementation with a spectral width of 6 kHz. Spectra were acquired with 512 data points in the t_1 direction and 2 K data points in the t_2 direction.[16,17] The time domain data were zero filled and multiplied with a shifted sine-bell function before Fourier transformation.

Statistical analysis. Differences in ratios of metabolites between the BPH and adenocarcinoma groups were investigated by the Kruskal-Wallis test at an experimental error rate of 0.05.

RESULTS

The results of the histopathological evaluation of the specimens used for PCA extraction were compared with the pathology reports and confirmed that representative samples of the tissues were investigated. All adenocarcinomas had a Gleason score higher than 8. Semiquantitative estimation of the percentage of tissue in the prostate cancers that could be evaluated showed more than 70% of tumor in two prostate cancer samples. In these two samples no signs of normal epithelial structures or BPH compounds were seen. One sample of prostate cancer consisted of 60% adenocarcinoma, no BPH and less than 5% normal epithelial structures.

One BPH sample consisted of almost 100% of glandular hyperplasia. In the other six samples the percentage of BPH was 40 to 50% with 10 to 20% normal epithelial structures.

It must be emphasized that the number of patients with BPH and adenocarcinoma of the prostate is small. Moreover, all tumors are in an advanced stage. Our results, as presented below, should therefore be considered as the first step in characterization of the metabolic content of the prostate and evaluation of the metabolic state of BPH and advanced prostate cancer.

^1H nuclear magnetic resonance spectroscopy. A 500 mHz proton MRS spectrum of a PCA extract of normal prostate tissue, with two expanded parts, is shown in figure 1. Numerous resonance peaks originating from protons of prostate compounds can be distinguished in the spectrum. Assignments for the methyl singlet peaks of phosphocholine (PC), choline (Chol) and (phospho)creatine ((P)Cr) were taken from the literature.[18,19] A number of other resonances were identified with the help of double-quantum filtered correlated (DQF-COSY) homonuclear ^1H-^1H spectroscopy (results not shown). Proton-proton connectivities on the COSY spectra were used for the assignment of lactate (Lac), alanine (Ala), citrate (Cit), taurine (Tau) and inositol (Ino). Many more connectivities were observed that may assist identification of other compounds. However, for this study we have restricted ourselves to an analysis of prostate compounds contributing to the most dominating resonances in the spectrum. These resonances are also expected to be important for in vivo MRS studies. For each compound the best resolved resonance(s) were selected for quantitation purposes. A signal of a stable homogeneously distributed compound would be ideal as an internal tissue standard for quantitation. Such a compound is not available for the prostate, and we have adopted the signals of total creatine (TCr) for quantification (creatine plus phosphocreatine is relatively stable during hypoxia and prolonged ischemia), but these may differ as a function of muscular content or tumor tissue.[20,21]

Table 1 lists relative peak area integral ratios involving the doublet of the CH3 group of lactate at 1.33 ppm, the doublet of the CH3 group of alanine at 1.49 ppm, the quartet of the CH2 group of citrate at 2.54 ppm, the singlet of the CH3 groups of (phospho)creatine at 3.04/3.05 ppm, the singlets of the CH3 group of phosphocholine and choline at 3.23 and 3.22 ppm, respectively, the triplet of the CH2 group of taurine at 3.42 ppm and the triplet of the CH group of inositol at 3.63 ppm.

In figure 2 representative ^1H MRS spectra are shown of PCA extracts of NP (C), BPH (B) and prostate cancer tissue (A). ^1H MRS spectra of BPH and the NP tissue PCA extracts were comparable for most ratios, whereas the ^1H MRS spectra of BPH showed marked differences in the relative peak ratios as compared with the ^1H MRS spectra of the prostate adenocarcinomas. Most striking is the presence of citrate in the spectra of the BPH specimens and the very low or undetectable levels in the specimens of advanced prostate carcinoma (table 1, $p < 0.05$). In one of the two cases in which citrate could be detected, normal epithelial structures were seen. In contrast, no citrate was observed in the prostate carcinoma biopsies containing no normal epithelial structures. In addition, a significant difference in the Ala/TCr, PC/TCr and Chol/TCr ratios between BPH and prostate cancer samples was observed (table 1, $p < 0.05$).

^{31}P magnetic resonance spectroscopy. Figure 3 shows a ^{31}P MRS spectrum of a PCA extract of normal prostate tissue. ^{31}P MRS resonances were assigned to phosphorylated compounds based on literature data.[22,23] Several phosphomonoester peaks, such as phosphoethanolamine (PE) and phosphocholine (PC), and phosphodiester peaks, such as glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC), as well as α, β and τ signals from different nucleoside triphosphate (NTP) peaks and inorganic phosphate (Pi) and phosphocreatine were identified and are indicated in figure 3. In table 2, ratios of