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In Vivo Proton MR Spectroscopy Reveals Altered Metabolite Content in Malignant Prostate Tissue

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Abstract. Background: Recently the potential of magnetic resonance (MR) methods for non-invasive diagnosis and therapy evaluation of prostate cancer has improved substantially. In this study proton MR spectroscopy (1H MRS) was explored for the detection of cancer in the prostate. Patients and Methods: Employing an endorectal probe localized 1H MRS and contrast enhanced MR imaging was performed on the prostate of healthy volunteers and of patients with benign prostatic hyperplasia (BPH) and/or prostate cancer (PCa). Results: 1H MR spectra of the human prostate showed major signals for citrate, creatine and choline compounds. For cancer tissue the average citrate/choline signal ratio was significantly lower than for BPH and non-cancerous peripheral and central zone tissue, but individual ratios overlapped with ratios for normal central zone and BPH tissue. Low citrate/choline ratios in tumour tissue correspond with early MR contrast enhancement. Conclusion: 1H MRS has potential for non-invasive detection and follow-up of tumours in the prostate.

It is common practice to verify the presence of cancerous tissue in the prostate by histology of biopsy material. However, this analysis may be subject to sampling errors [1] and does not provide much information on spatial heterogeneity and multifocality. Non-invasive modalities such as computer tomography and transrectal ultrasound (without guided biopsies) have been of limited value for diagnosis and therapy evaluation of prostate cancer [2]. Also MR imaging (MRI) is of little use to detect PCa and to determine its extent, although the more recent introduction of endorectal and body phased-array radiofrequency detection coils have enabled better staging of PCa [3,4,5]. The potential of MR may be further improved by the use of more advanced methods such as dynamic Gd contrast enhanced MRI or MR spectroscopy (MRS). Several groups have started to perform image-guided proton MRS (1H MRS) of the human prostate with endorectal and/or phase d-array coils [6-9]. In a 1H MRS examination spectra can be obtained of particular locations in the prostate with proton signals of several metabolites. In this way it provides a metabolic window on prostate tissue which may be used for in situ characterization, diagnosis and therapy evaluation of prostate cancer (PCa).

The most prominent metabolite signal in 1H MR spectra of the prostate is that of citrate, of which the tissue content has been reported as a potential marker to discriminate between the presence of PCa and benign prostatic hyperplasia (BPH) [10-16]. In vitro studies of human prostate specimens have demonstrated that the relative intensity of the methyl proton signals of (phospho)choline compounds in 1H MR spectra of PCa tissue is increased [15,16] and our preliminary results indicated that this could also be observed in vivo [8].

In this paper we report on results of the application of image-guided localized 1H MRS in studies of the prostate of healthy volunteers, patients with BPH and/or with PCa. We applied and compared single and multiple voxel data acquisition methods. As elevation of choline and lowering of citrate levels appear to be associated with PCa, the citrate/choline (Ci/Ch) signal ratio plays a central role in the analysis of our data. Preliminary results on the combination of 1H MRS and dynamic contrast enhanced Gadolinium (Gd) MR measurements are also presented.
Patients and Methods

Volunteers and patients. This study was approved by the local ethical committee and informed consent was obtained from all participants. Results of studies of 6 healthy volunteers aging from 32 to 54, without a history or clinical signs of prostate disease, are included in this report. MR images of the prostates were interpreted as being normal except for some slight hypertrophy of the oldest volunteer. Sixteen patients with BPH were included in this study (ages between 52 and 73). Furthermore, 23 patients suspected to have PCa, mainly on the basis of PSA levels (10 ng/ml or more) and ultrasonography, were investigated. The definitive diagnosis (PCa or not) was made on the basis of histological analysis performed on prostate tissue specimens obtained by needle biopsy or radical prostatectomy. Subjects were positioned supine in the magnet bore with a belt applied around the lower abdomen to reduce respiratory motion. Patients received 1 mg glucagon intravenously as antiperistaltic drug.

MR methods. MR examinations were performed on a 1.5 T MR system (Magnetom SP, Siemens, Germany) employing a body radio-frequency coil for excitation. For MR signal reception a disposable endorectal probe (MEDRAS) was inserted. The probe was inflated with air to ensure tight positioning of the coil adjacent to the prostate.

MRI and MRS of the prostate was performed during the same patient examination which lasted about 1 hour. First multiple slice MR images were obtained in 3 orthogonal planes with a turbo spin-echo (TSE) sequence as described previously [5]. Guided by these images the prostate was inspected for the presence of areas likely to contain cancer tissue (hypo-intensity in the peripheral zone and anatomical deviations). Localized 1H MRS was performed with a double spin-echo slice selective pulse sequence (PRESS [17,18]) preceded by water signal suppression which first was turned off to optimize field homogeneity. The echo-time (TE) was 135 ms.

For single voxel MRS a voxel of nominal 1.5*1.5*1.5 cm (3.4 cc) was positioned in an area suspected to be tumour tissue, and if possible another voxel was studied in a non-cancerous area. The scan repetition time (TR) ranged from 1.6 to 4.5 seconds and the number of scans from 96 to 256.

In the 1H MR spectroscopic imaging (SI) experiments an axial slab of 10 mm thickness, 40 (or 50) mm in the left-right and 30 (or 40) mm in the anterior-posterior direction was selected using the PRESS sequence (TR = 120 - 135 ms) and positioned such that it contained a maximal amount of area suspected to be cancer tissue. Gradients for phase encoding in 2 directions were applied to obtain a multiple voxel SI data set within this preselcted slab [19,20]. Data were encoded in a 16 * 16 matrix with a field-of-view of 144 or 160 mm, resulting in nominal voxel sizes of 0.8 or 1.0 cc. Per phase-encoding step 2 or 3 acquisitions were acquired with a TR of 1.2 or 1.6 seconds resulting in acquisition times of 13 - 15 minutes.

For eddy current correction and referencing purposes MRS experiments were also performed without water suppression.

In a number of patients suspected of having PCa a dynamic Gd contrast enhanced inflow MR measurement was performed [21,22] after the MRS measurements. An axial slice of 10 mm thickness was selected through the suspected tumour region at exactly the same location as the SI slab or coinciding with the position of the single voxel. MR images were obtained by a turbo FLASH sequence (1 - 2 seconds/image) before and during inflow in the slice of Gd contrast, which was administered intravenously.

Post-processing was performed as described elsewhere [23]. Metabolite maps were reconstructed from the intensities of citrate and choline proton signals in SI spectra, showing the spatial distribution of these compounds. Chemical shifts are given in parts per million (ppm) with respect to the water resonance at 4.68 ppm. Signal integrals were determined by computer integration. All presented signal ratios for citrate and choline were corrected for T1 weighting using an effective T1 of 339 ms for citrate protons and of 837 ms for choline protons as determined in a separate volunteer study [23]. Average values are presented with standard deviations (±SD).

Dynamic Gd contrast enhanced MR experiments are presented as dynamic subtraction (DS) images obtained by subtracting MR images recorded during Gd inflow in the slice from control images recorded before inflow.

Results

In Figure 1A a T2 weighted MR image is shown of an axial plane through the prostate of a patient with PCa. An enlarged central gland (BPH) can be observed as a hypo-intense area including some hyper-intense nodular elements. The peripheral part of the prostate shows up as a hyper-intense zone except at the right side on this image where it is hypo-intense (solid box). This area was later demonstrated to be tumour tissue by histological examination. It showed earlier enhancement than other prostate tissue on DS MR images (see arrow in Figure 1B). 1H MRS was performed for voxels (see Figure 1A) of which the spectra are shown in figures 1C and D. The spectrum in Figure 1C, from a region assumed to house no cancer tissue, looks similar to spectra obtained from the peripheral zone of normal and most BPH prostate. It shows 3 major resonances. One at about 2.6 ppm originating from the methylene protons of citrate, one at about 3.0 ppm originating from the methyl group of creatine and one at about 3.2 ppm to which methyl groups of various choline containing compounds contribute. These assignments are based on previous 1H MRS studies of perchloric extracts of prostate specimens [12-16]. The spectrum from the tumorous region (Figure 1D) shows a strongly reduced citrate signal and an apparently increased choline and decreased creatine resonance.

Fifteen patients suspected of having PCa were examined by this single voxel approach. Two patients declined further medical examination after the MR investigation so that no definitive diagnosis is available and 3 were considered to have BPH after multiple biopsies. In 10 cases the presence of PCa was proven by histopathological examination of prostate material, either obtained by biopsy or radical prostatectomy [5]. After careful evaluation of the MR images and comparison with the histopathological results it was concluded that in 3 of these cases the voxels, with the aim of covering cancer tissue, were either located in an area with no apparent presence of cancer tissue or were mostly occupied with non-cancerous tissue (> 75 %). For the 7 remaining cases it was estimated that the voxels mainly contained tumour tissue (> 50 %). The citrate/choline signal ratio was determined from the spectra for further evaluation (see below).

In 3 patients suspected of having PCa a dynamic Gd MRI measurement was performed after the single voxel 1H MRS

1456
A. T2 weighted axial TSE image of the prostate. Boxes indicate the size and location of two selected voxels for 1H MRS.
B. 1H MR image showing focal enhancement (arrow) due to Gd contrast inflow in the tumor. The image is obtained at approximately 12 seconds after the start of inflow in the imaged slice.
C. 1H MR spectrum of the volume indicated by the dashed box.
D. 1H MR spectrum of the volume indicated by the solid box (tumor area).
Ch=Choline compounds; Cr=creatine; Cl=citrate.

Figure 1. Results of an MR examination of a patient with PCa (T3c; PSA 37 ng/ml).

Figure 2a and b show T2 weighted MR images of an axial slice. Metabolic maps, reconstructed from signals of the SI data set are overlaid on these images. On the MR image a hypo-intense area in the peripheral zone is visible (arrow) extending into the prostate which was later identified as a dynamic Gd MR examination are shown of a PCa patient. Eight patients suspected of having PCa were investigated by 2D SI in an axial slab through the prostate using similar experimental parameters as for the single voxel measurements. In figure 2 the results of a 2D SI and a
cancer tissue. The citrate metabolite map (Figure 2a) shows a relative low citrate level in this area. In contrast the choline metabolite map (Figure 2b) shows increased intensity. Furthermore, DS MR images from a dynamic Gd MR investigation of the same slice showed fast enhancement in this area (Figure 2c).

Of the 8 patients suspected of having PCa and investigated by SI, 5 were finally diagnosed as having PCa on the basis of biopsy material or radical prostatectomy specimens [5]. For 1 patient no final diagnosis could be obtained, while histopathological examination of biopsy material from 2 other patients showed no apparent malignancy. From the five SI data sets voxels were selected in identified cancerous areas and in areas assumed not to be affected by cancer. Ci/Ch signal ratios were determined from the spectra of these voxels.

Although generally the experiments indicate increased choline signals in tumorous regions, in some cases the choline signal actually seemed to be decreased in such regions (with respect to the signal of water as an internal reference), but because the citrate signal decreased much more, the Ci/Ch signal ratio still served as a potential marker of malignancy.

The plot in Figure 3 summarizes the evaluation of the Ci/Ch signal ratio derived from $^1$H MR spectra of normal
prostates, BPH and PCa patients. It shows the average value of this ratio and standard deviations obtained from SI experiments of the normal prostates of healthy volunteers ($n=6$). The Ci/Ch signal ratio value ranges from 2.2 to 5.4 for the peripheral zone and from 0.6 to 1.9 for the central zone. For BPH patients ($n=14$) the Ci/Ch signal ratio was determined from single voxel measurements with mainly central gland tissue (BPH region) and shows a wide range of values: from 0.8 to 7.6. The range of values in non-tumour areas of peripheral zones of PCa and BPH patients determined with SI is from 1.7 to 3.5 ($n=7$).

The average Ci/Ch signal ratio obtained from all single voxel plus SI spectra from tumour regions ($n=12$) is significantly lower compared with the other groups: peripheral zone, central zone and BPH regions (t tests: $p < 0.002$ for each comparison). However, the range of individual values (1.2-1.2) overlaps with the ratios obtained from central zone tissue in normal prostates and from BPH tissue.

**Discussion**

Citrate is highly abundant in the normal human prostate and mainly resides in the luminal space of the prostate [10]. For the normal prostate citrate signal levels are higher in $^1$H MR spectra of the peripheral zone than in spectra of the central gland (including the transition zone and periurethral tissue) [9,23,24]. This is also evident in this study and is related to the different amount of citrate secreting elements in both parts of the prostate. The large variation of relative citrate signals for central gland BPH tissue is most likely caused by differences in glandular and stromal contributions. This study shows that decreased relative levels of citrate in cancer tissue of the prostate can be detected by in vivo $^1$H MRS. Lower citrate levels found in cancerous tissue are presumably due to a reduction of citrate secreting epithelial structures [10,14,15].

Results from this and previous studies [8,15,16,24] indicate that the choline resonance is increased in cancerous areas of the prostate, but occasionally we have also observed reduced relative choline signals for PCa tissue in vivo. Elevated signals for choline compounds have been reported from $^1$H MRS studies of brain tumours [25] and are assumed to be related to a high proliferation rate of tumour cells.

The average $T_1$-corrected citrate/choline signal ratio, which we used for quantitative purposes, appears to be significantly reduced in tumour tissue compared to normal prostate and BPH tissue. However, as there is overlap with values of BPH tissue and the normal central zone at the present status of MRS, the potential clinical applicability is restricted to peripheral zone tumours. The results of this study generally are in good agreement with results published recently by Kurhanewicz et al [24] employing a similar MRS approach in 3 dimensions.

One cause of the failure to place the volume of interest in a malignant region of the prostate by single volume MRS in a number of cases is due to the fact that conventional MRI is not very reliable in depicting prostate tumours. This shows that a multiple volume method for MRS enabling spatial evaluation, as efficiently provided by SI methods, is essential. Preferentially, spectra should be obtained from voxels covering the whole prostate at high spatial resolution [24].

This study also demonstrates that $T_2$-weighted MRI, $^1$H MRS and dynamic Gd enhanced MRI can be combined in a single patient examination. Dynamic Gd enhanced MRI has proven to be successful in the detection of some malignancies [21]. This is based on the specific (transient) accumulation of the contrast compound in tumour tissue related to its typical neo-vascular characteristics. Preliminary results indicate that it also improves prostate tumour detection (26). $^1$H MRSI in combination with dynamic Gd contrast enhanced MRI may be helpful to improve the characterization of cancer in prostate tissue.

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