16T Diffusion Microimaging of Fixed Prostate Tissue. Preliminary Findings

Abstract

The study reported here used diffusion tensor microimaging (µDTI) to investigate the water diffusion properties of formalin-fixed prostate tissue at spatial resolution approaching the cellular scale. Prostate tissue was collected from a formalin fixed radical prostatectomy specimen with a 3mm core punch. µDTI was performed at 16.4T with 40µm isotropic voxels. µDTI clearly demonstrated distinct microscopic diffusion environments and tissue architecture consistent with that seen on light microscopy of the same tissue. The most restricted and isotropic diffusion environment is the secretory epithelial cell layer (voxel bulk mean diffusivity, $D = 0.4 \pm 0.1 \times 10^{-3} \text{ mm}^2/\text{s}$, FA = 0.32± 0.15). Diffusion in the fibromuscular stromal matrix is relatively less restricted but more anisotropic (D = 0.7 ± 0.1 $\times 10^{-3} \text{ mm}^2/\text{s}$, FA = 0.61± 0.15). Allowing for partial volume effect, the actual bulk mean diffusivity in the epithelial cell layer was estimated to be 0.2-0.3 $\times 10^{-3} \text{ mm}^2/\text{s}$. In tumor tissue (Gleason pattern 4+4) distinct glandular and ductal structures are absent in the diffusion-weighted diffusion-weighted images (D = 0.5 ± 0.1 $\times 10^{-3} \text{ mm}^2/\text{s}$; FA = 0.35± 0.11).

Distinct stromal and epithelial diffusion compartments are the most likely origin of biexponential diffusion decay observed in vivo.

Introduction

Since prostate cancer is defined by tissue structure seen at histopathology, a detection method that generates contrast based on microscopic tissue structural properties might be expected to provide both sensitive and specific cancer detection to the extent that the contrast can be made to reflect the structures that define pathology. Diffusion-weighted water imaging (DWI) is an obvious candidate for this purpose since the free diffusion of water in tissue is known to be constrained by intra- and extracellular structures and cell walls. In contrast to $T_1$ and $T_2$ weighted water imaging, DWI can reveal both the scale and orientation of tissue structure because DWI contrast depends on the net diffusion of water over a specific time period in a specific direction. Two parameters are commonly used to describe the rate and spatial freedom of water diffusion - the apparent self diffusion coefficient (ADC), and the fractional anisotropy (FA), respectively (1). In response to inconsistent reported definitions of ADC Basser & Jones (year?) have recommended the use of the bulk mean diffusivity (D) to describe the directionally averaged freedom of water diffusion (1).

In vivo DWI studies of prostate tissue have generally demonstrated a decrease in the measured ADC in prostate cancer tissue relative to normal PZ glandular tissue. However, in the heterogeneous central zone (CZ) assignment of diffusion characteristics to tissue type has been equivocal. Attempts to measure diffusion anisotropy (of which exactly?) in vivo have produced equivocal results (2-7).

In vivo imaging suffers from poor spatial resolution, movement and susceptibility artifacts, and extreme difficulty in accurate correlation of imaging data with tissue type or pathologic status. Xu et al. (8) obtained reliable correlations between diffusion measurements and tissue type and
pathology from a study of formalin fixed radical prostatectomy specimens. Their study, performed at 4.7T with spatial resolution 0.5 x 0.5 x 0.5 mm, obtained diffusion anisotropy data consistent with gross tissue architecture. High FA was observed in regions of primarily fibromuscular stromal tissue with the primary diffusion axis (principle eigenvector) parallel to the assumed main fiber axis. Xu et al. suggested that the observed decrease in diffusivity in cancer relative to normal glandular tissue was consistent with: 1) the loss of large luminal and ductal spaces (typically 100-200µm in diameter) in which water diffusion would be expected to be relatively unrestricted relative to the intracellular and intercellular environment of packed cells; and 2) increased cell density characteristic of prostatic adenocarcinoma (9).

Very high spatial resolution diffusion MRI can potentially eliminate or substantially reduce the partial volume effects that compromise the interpretation of results from lower resolution ex vivo studies of fixed tissue and in vivo studies of intact tissue. The study reported here used 16.4T diffusion microimaging with high diffusion gradient strength to characterize the water diffusion properties of fixed prostate tissue at spatial resolution approaching the cellular scale.

Methods

Tissue collection. Prostate tissue was collected from a radical prostatectomy specimen. The whole organ, immersed 72hr in 10% neutral buffered formalin (NBF) post surgery, was sectioned for routine histopathology. Five millimeter transverse slices were examined by a specialist urologic pathologist and tissue samples obtained with a 3mm core punch. The selection of regions for sampling was at least 5mm internal to the prostatic capsule and based on visual assessment of the likely tissue type. Cores were placed in vials of 10% NBF and stored 4 weeks at room temperature prior to MR imaging after which the cores were returned for histopathology. All tissue samples were collected with institutional ethics approval and written informed consent from the tissue donor.

MR microimaging. Tissue cores were transferred from NBF to phosphate buffered saline (PBS) containing 0.2% v/v gadolinium contrast agent (Magnevist, Schering AG, Germany). Dimethylamine gadopentetate 4.69 g/10ml and stored overnight at room temperature to wash out formaldehyde. Cores were then removed from contrast/PBS and glued (cyanoacrylate “Superglue”) to a plastic strip to constrain the position of the core during imaging. The core and plastic strip were inserted into a 5mm diameter NMR tube filled with contrast/PBS solution for imaging. The first sample (cancer tissue) was immersed in perfluorocarbon in order to minimize the background signal, however, after observing penetration of the perfluorocarbon into the tissue we desisted with its use and immersed later samples in contrast/PBS.

Imaging was performed on a Bruker (Germany) AV700 magnetic resonance microimaging system consisting of a 16.4 T vertical bore magnet interfaced to an AVANCE II spectrometer running Paravision 4 and using a 5 mm solenoid RF coil. The scanner was equipped with a Micro2.5 gradient set (2.5 G/cm/A). Imaging was performed at room temperature (22 °C). The high resolution T2 *-weighted imaging protocol was a 3D gradient echo sequence with the following parameters: TR = 40 ms, TE = 6 ms, pulse angle = Ernst angle (~30°), number of averages = 8, total imaging time = 4 hr 30 min, FOV = 8 x 4.5 x 4.5 mm, acquisition matrix = 400 x 224 x 224, zero filled to give an image matrix of 512 x 256 x 256 for an image resolution of 15.6 x 17.6 x 17.6 µm (raw data resolution = 20 x 20 x 20 µm).
For diffusion weighted imaging a 3D spin echo DTI sequence with the following parameters was used: TR = 500 ms, TE = 17.9 ms, number of averages = 1, total imaging time = 14 hr, FOV = 8 x 4.5 x 4.5 mm, acquisition matrix = 200 x 112 x 112 (raw data resolution = 40 x 40 x 40 µm).

Diffusion parameters: δ = 2 ms, Δ = 12 ms, b=1500 s/mm² (37% gradient power), with six non-collinear directions and two b=0 images.

Diffusion parameters were calculated using the program DiffusionToolkit (www.trackvis.org, Ruopeng Wang and Van J. Wedeen. TrackVis.org, Martinos Center for Biomedical Imaging, Massachusetts General Hospital, USA). The resulting images were displayed and analysed with the program MIPAV (Version 0.4.4. mipav.cit.nih.gov, Centre for Information Technology, NIH, USA). Volume rendering was performed with MIPAV (Version 4.3.1). Voxel parameter values were measured by manual drawing of regions of interest in MIPAV. Reported diffusion parameters are:

*Mean diffusivity* \(D = (\lambda_1 + \lambda_2 + \lambda_3)/3\) where \(\lambda_1, \lambda_2, \lambda_3\) are the eigenvalues of the diffusion tensor matrix.

*Mean squared displacement* \(MSD = 6D (\Delta - \delta/3)\) where \(\Delta\) is the time interval between diffusion gradient pulses of duration \(\delta\).

*Fractional anisotropy*

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FA = \frac{\sqrt[3]{(\lambda_1 - \bar{\lambda})^2 + (\lambda_2 - \bar{\lambda})^2 + (\lambda_3 - \bar{\lambda})^2}}{\sqrt[2]{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}}
\]

where \(\bar{\lambda}\) is the mean of the eigenvalues.

**Histopathology.** Tissue was returned from imaging still glued to plastic strips. The tissue surface was marked with ink according to the MR imaging planes, carefully excised from the plastic strip, and then processed normally. The ink marking was used to embed the tissue such that sectioning planes were approximately parallel to the MR imaging planes.

**Results**

**Diffusion compartmentation.**

Fig. 1 demonstrates that the microscopic structure of normal glandular prostate tissue is clearly visible in diffusion-weighted microimages but is not distinct in \(T_2^*\)-weighted images. The 3D structure of normal glandular tissue is demonstrated in a surface rendered volume (Fig. 2). Three distinct diffusion compartments are apparent: ductal lumen, stromal matrix, and epithelium and these correspond closely to the glandular architecture seen on light microscopy.
Fig. 1. Comparison of T2*-weighted (A) and diffusion-weighted (B) images of fixed prostate tissue. Note that the epithelial cell layer is distinctly hyperintense in the diffusion-weighted images indicating highly restricted water diffusion in this layer. Scale bar = 1mm.

Fig. 2. Surface rendered diffusion-weighted volume image of normal prostate tissue clearly illustrates ductal structures lined with secretory epithelia embedded in the stromal matrix. Three distinct diffusion compartments are apparent: ductal lumen, stromal matrix, and epithelium. (where are these in the image?)

Correlation of diffusivity with cell type.

In normal glandular tissue water diffusion appears highly restricted in voxels containing the epithelial cell layer ($D = 0.4 \pm 0.1 \times 10^{-3} \text{ mm}^2/\text{s}$, corresponding to a mean net displacement of $\sim 5\mu\text{m}$ during the 12ms diffusion measurement period). Diffusivity in the ductal lumen ($D = 2.1 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$) is similar to that observed in the contrast/PBS solution outside the tissue sample. Intermediate diffusivity is seen in the fibromuscular stromal matrix ($D = 0.7 \pm 0.1 \times 10^{-3} \text{ mm}^2/\text{s}$). In the tumor tissue sample (Gleason pattern 4+4 adenocarcinoma) normal glandular structures were not seen in the diffusion images and the tissue has a low ($D =$

Comment [NK4]: What about correlation with the images from histological slices? Are we seeing the same observation as Xu et al.?
0.5±0.1 x 10^{-3} \text{ mm}^2/\text{s}) and homogeneous diffusivity consistent with extensive proliferation of epithelial cells.

**Diffusion anisotropy**

The calculated FA (Fig. 3) shows higher average anisotropy in voxels of primarily fibromuscular stromal tissue (FA = 0.61± 0.15) than in those containing secretory epithelium (FA = 0.32± 0.15). Apparently high measurements of FA can be a product of low SNR (5) as is evident in the saline solution surrounding the tissue and in ductal lumen spaces where FA was expected to be zero. In the restricted diffusion environment of the epithelial cell layer the SNR is high and the low FA values are unlikely to be artificially high due to noise. It is clear that FA is consistently lower in epithelial cells than in the surrounding fibromuscular matrix. Also, more pixels in regions of fibromuscular matrix have high FA values than in the low SNR solution surrounding the tissue sample, suggesting that the high FA values seen in fibromuscular tissue are not solely due to noise.

**Discussion**

This is the first report of diffusion-weighted MRI of glandular tissue with a resolution that approaches the cellular scale. The 40µm isotropic voxel volume (approx. 20 cell volumes) is more than three orders of magnitude smaller than that used in previous high resolution studies of prostate tissue (8,10). This high spatial resolution permits investigation of the tissue diffusion properties with minimal partial volume effects.

**Diffusion compartmentation**

The diffusivities we measured are generally within the range found in the lower spatial resolution studies (0.5x0.5x0.5mm) of formalin fixed prostate tissue by Xu et al (8). The average voxel diffusivity in one full slice of normal glandular tissue (0.7 x 10^{-3} \text{ mm}^2/\text{s}) was at the low end of the range Xu et al. reported for “benign PZ”.

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*Comment [NKS]: is it worth to mark in the figures, the structures that you mentioned in the text with *,# Also you made a few references to saline, I don’t know where it is in the image?*
A number of in vivo prostate tissue studies have demonstrated that when a large range of b values are measured (up to 3000 s/mm²) the diffusion-weighted signal shows a biexponential decay (11-13). The physical basis of the biexponential decay has been hypothesized to result from a range of factors including free and restricted diffusion compartments, exchange between such compartments, T₂ relaxation effects (14), and macromolecule binding (13). Our results strongly suggest that large differences between mean diffusivities in the ductal spaces, the epithelial cell layer, and the stromal matrix may be the major contributor to biexponential behavior observed at low spatial resolution. Preliminary medium resolution measurements (140µm isotropic voxels) in fixed tissue with 16 b values from 0-1000 s/mm² produced good biexponential fits with component diffusivities that cluster around D values of 0.4 and 0.8 x 10⁻³ mm²/s – consistent with the measured diffusivities in the epithelial and stromal compartments. The absence of a significant contribution from the high diffusivity ductal compartment may be due to its small partial volume in our samples (Fig. 2).

We observed that the Gleason pattern 4+4 prostate cancer tissue sample had generally lower diffusivity than stroma, with D similar to that in epithelium-containing voxels of the normal glandular tissue sample. This suggests that the observed diffusivity in cancer tissue is primarily a characteristic of diffusion within epithelial cells rather than in the extracellular space and is consistent with the decreased extracellular volume fraction required for an optimal biexponential fit to signal decay in cancer tissue in vivo (13).

**Diffusion anisotropy**

Our estimates of FA in stromal tissue are limited in accuracy and probably high due to low SNR in this compartment at b=1500 s/mm² (5). However, it is clear that FA is higher in stromal tissue than in the epithelial cell layer. In this respect our data explains the earlier low spatial resolution finding that primarily stromal regions had higher FA than regions dominated by epithelial cells (8).

**Limitations**

Formalin fixation, as used for tissue stabilization in this study, results in extensive cross-linking of tissue protein. The expected consequent decrease in water diffusivity relative to unfixed tissue has been previously observed in prostate tissue although relative diffusivities of different tissue types were only slightly affected (8). This suggests that fixed tissue remains a useful model for elucidation of water diffusion behavior in vivo.

**Implications**

Diffusion microimaging has demonstrated the likely origin of biexponential diffusion decay observed in in-vivo prostate MRI. Given the close correlation between diffusion compartmentation and glandular tissue structure, biexponential diffusion parameter mapping may improve the sensitivity and specificity of MRI-based detection and grading of prostate cancer.
References


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