Biexponential Diffusion Decay in Formalin Fixed Prostate Tissue: Preliminary Findings

Authors:

Roger M. Bourne[1], Nyoman Kurniawan[2], Gary Cowin[2], Timothy Stait-Gardner[3], Paul Sved[4], Geoffrey Watson[5], Supriya Chowdhury[6], and William S. Price[3]

1. Discipline of Medical Radiation Sciences, Faculty of Health Sciences, University of Sydney
2. Center for Advanced Imaging, University of Queensland.
3. School of Biomedical and Health Sciences, University of Western Sydney.
4. Department of Urology, Royal Prince Alfred Hospital, Sydney and University of Sydney
5. Department of Anatomical Pathology, Royal Prince Alfred Hospital, Sydney
6. Sydney Medical School, University of Sydney

Corresponding author:
Dr. Roger Bourne,
Discipline of Medical Radiation Sciences,
Faculty of Health Sciences,
University of Sydney,
PO Box 170, Lidcombe, 1825
Australia.
email: roger.bourne@sydney.edu.au
Fax: +61-2-9351 9146

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Abstract

Magnetic resonance microimaging was used to measure diffusion decay over an extended *b*-factor range in a formalin fixed normal prostate sample and a Gleason pattern 3+4 tissue sample. The coefficients of biexponential fits to diffusion decay data from 1600 voxels of dimension 160×160×160 μm^3 in each sample were correlated with underlying epithelial and stromal compartment partial volumes estimated from high resolution ADC data (40×40×40 μm^3 voxels) from the same tissue. In the normal tissue sample the signal fractions of the low and high ADC components of the biexponential fits correlated linearly with partial volumes of epithelial (R^2 = 0.6) and stromal tissue (R^2 = 0.5) respectively. Similar but weaker correlations were observed in the cancer sample. Epithelium-containing high spatial resolution voxels appeared to be comprised of ~60% low ADC and ~40% high ADC component. Stromal voxels appeared to be comprised of ~20% low ADC and ~80% high ADC component. This preliminary report suggests that distinctly different diffusion properties in microscopically adjacent cell types contribute to the multiexponential diffusion decay phenomenon.

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Keywords: Diffusion decay, prostate, compartmentation, multiexponential, biexponential

Introduction [can be shortened]

A profile of tissue specific multiexponential signal decay (mESD) can be observed ~~When~~ when diffusion weighted MRI (DWI) is performed over an extended range of *b*-values ~~multiexponential signal decay (mESD) is observed in a wide range of biological tissues~~. It has been suggested that an mESD analysis of prostate DWI might provide higher specificity for detection of cancer than current methods based on the apparent diffusion coefficient (ADC) calculated from a monoexponential model (1-2).

The ubiquity of the mESD phenomenon in both biological and non-biological systems is accompanied by a plethora of hypotheses about, and investigations of, its biophysical origin. ~~References~~ (3-4) ~~provide theoretical background~~. In biological tissue hypotheses about the origin of mESD commonly involve the compartmentation of water in physically separate microenvironments with distinct diffusion properties, with or without exchange between the compartments. When mESD appears ~~essentially~~ biexponential the two compartments assigned to the slow and fast diffusing components are most commonly assumed to be "intracellular" and "extracellular" respectively. However, several ~~elegant~~ studies of mESD ~~in vitro~~, using variable density suspensions or immobilizations of single cells, suggest that a simple intra/extracellular model is overly simplistic (5-6). For example, mESD has been reported from the cytoplasm of a single cell (7).

In prostate tissue there is both indirect and direct evidence for the presence of distinct microscopic diffusion compartments. Indirect evidence comes from reports of multiexponential DWI signal decay ~~in vivo~~in-vivo (1-2). As well as free and restricted diffusion compartments, multiexponential decay has been hypothesized to result from other factors including exchange between restricted diffusion compartments (8), T_2 relaxation effects (9), and macromolecule binding (2).

Recently, direct evidence of three distinct diffusion compartments has come from MR microimaging studies of formalin fixed prostate tissue (10-11). These studies demonstrated highly restricted diffusion in voxels containing the epithelial cell layer, intermediate diffusivity in the stromal matrix, and free or slightly restricted diffusion in ducts and acinar lumena. There was a close correlation between structural features visible in DWI and tissue architecture seen on light microscopy of the same tissue. Due to the apparently small total partial volume of ductal space the authors suggested that microscopic diffusion compartmentation between the epithelial and stromal tissues was the likely origin of biexponential diffusion decay observed at low spatial resolution ~~in vivo~~in-vivo.

H~~The study reported h~~ere ~~uses~~ diffusion microimaging was used to investigate the relationship between microscopic diffusion compartmentation and mESD in prostate tissue. Our experimental hypothesis was that the coefficients of multiexponential diffusion signal decay measured at medium spatial resolution could be explained by microscopic diffusion compartmentation measured in the same tissue at high spatial resolution.

Methods

Tissue collection.

Tissue samples were collected from radical prostatectomy specimens with ~~institutional~~ ethics approval and ~~written informed~~ donor consent ~~from donors~~. A sample of normal glandular tissue was obtained from a ~~60 year old~~60-year-old patient with PSA 6 at diagnosis and Gleason pattern 3+4 cancer. Gleason pattern 3+4 cancer tissue was obtained from a second ~~60 year old~~60-year-old patient with PSA 5.8 at diagnosis. ~~Whole~~ The whole organs were~~,~~ immersed ~72 h in 10% neutral buffered formalin (NBF) post surgery~~,~~ and ~~were~~ sectioned for ~~routine~~ histopathology. Transverse slices (~4 mm thick) were examined by a specialist urologic pathologist and full thickness tissue samples were obtained from the lateral peripheral zone with a 3 mm core punch (sample volume ~28 mm^3). The pathologic status of these ~~tissue~~ samples was established by histopathologic examination of tissue immediately surrounding the core site and was consistent with the appearance of diffusion-weighted microimages. ~~Cores~~ The cores were ~~placed in vials of~~ stored in NBF ~~and stored~~for 2-10 weeks at room temperature prior to MR ~~I~~imaging.

MR microimaging.

Tissue cores were transferred from NBF to phosphate buffered saline (PBS) containing gadolinium contrast agent ([Gd] = 0.16 mM)(11). Imaging was performed at room temperature (22 ^oC) on a Bruker (Germany) AVANCE II ~~AV700 magnetic resonance microimaging system consisting of a~~ 16.4 T vertical bore magnet ~~interfaced to an AVANCE II spectrometer~~ running Paravision 5 and using a 5 mm solenoid RF coil and Micro2.5 gradient set.

To investigate the relationship between multiexponential diffusion decay and microscopic compartment diffusivity, medium and high spatial resolution diffusion data were acquired with a fixed field of view (FOV = 8×4.5×4.5 mm^3) and matrix sizes differing by a factor of four in each dimension. High spatial resolution data (40×40×40 μm^3) were acquired with three orthogonal diffusion directions using a 3D spin echo sequence with the following parameters: TR = 500 ms, TE = 18 ms, number of averages = 1, total imaging time = 9 h, acquisition matrix = 200×112×112. Diffusion parameters: δ = 2 ms, Δ = 12 ms, b = 1.2 ms/μm^2, one b = 0 image per slice. The intrinsic signal-to-noise ratio (SNR) calculated from b = 0 images was ~24 (12). Apparent diffusion coefficient (ADC) images were calculated from the geometric averages of the b = 0 and b = 1.2 ms/μm^2 images.

Medium spatial resolution (160×160×160 μm^3) multi-b diffusion decay data were acquired with the same FOV and diffusion imaging parameters as above with three orthogonal diffusion directions and eight effective b values of 0.032, 0.061, 0.126, 0.271, 0.566, 1.176, 2.397, and 4.897 ms/μm^2 ~~(TR = 500 ms, TE = 18 ms, number of averages = 1~~, total imaging time = 3 h, acquisition matrix = 50×28×28~~. Diffusion parameters: δ = 2 ms, Δ = 12 ms,~~ and two b = 0 images~~)~~. The intrinsic SNR~~signal to noise ratio~~ calculated from b = 0 images was ~174 (12). Each medium resolution voxel could thus be matched to 4×4×4 = 64 high resolution voxels for estimation of underlying fractional compartment volumes.

Analysis.

The image data were ~~displayed and~~ analyzed with Matlab (Mathworks, Natick, MA). Gaussian curve fitting to ADC histograms (11) was performed with the Matlab curve fitting tool (Method: non-linear least squares. Robust: On. Algorithm: Trust-region).

For diffusion decay analysis a 1.6×1.6×2.5 = 10.2 mm^3 "tissue only" subvolume of each sample was selected, comprising 10×10×16 = 1600 voxels of dimension 160 μm^3 each. This subvolume

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was centered in the region of maximum coil sensitivity and was wholly contained within the boundaries of the cylindrical tissue core. The subvolume thus contained only epithelium, stroma, and ductal space.

For each of the eight non-zero *b*-values DW images were calculated by averaging of the images from the three diffusion directions. For each of the 1600 voxels a biexponential fit was performed as above with code derived from the Matlab curve fitting tool ~~(method as above)~~ according to the equation:

$$I = A_1 e^{-ADC_1 \times b} + A_2 e^{-ADC_2 \times b} \qquad \text{(Eq. 1)}$$

where A_x is the size of the population of spins with apparent diffusion coefficient ADC_x.

We refer to the ratio $SF_x = A_x/(A_1 + A_2)$ as the *signal fraction* of component *x*. For each voxel fit the parameters A_1, A_2, ADC_1, ADC_2, and the R^2 "goodness of fit" were recorded. For the 1600 fits to the "tissue only" voxels 95% of the normal sample voxels and 98% of cancer sample voxels returned R^2 values greater than 0.9995. Fit coefficients were sorted by signal fraction such that the parameters A_1 and ADC_1 represent the major signal component in each voxel (ie. $A_1 \geq A_2$).

Results and Discussion

Effect of noise on biexponential fitting

Gaussian noise in quadrature detected signal acquisition results in the presence of a Rician "noise floor" in magnitude MR images. At SNR = 0 the noise has a Rayleigh probability density function (PDF), however, asymmetry of the noise PDF decreases with increasing SNR, and at SNR > 6 the noise PDF is effectively~~ssentially~~ Gaussian and the mean of the noisy signal is essentially identical to the noise-free signal (13).

For the 160 µm isotropic voxel data used for diffusion decay measurements we estimated intrinsic SNR_{160} = 174 (calculated according to (12)). This SNR implies that the noise floor would have a Gaussian PDF and thus that the non-linear least squares fitting method was reliable for the biexponential analysis (14). To estimate the reliability of the biexponential fits we performed Monte Carlo simulations based on a model with intrinsic SNR = 174, components with ADC and signal fractions equal to the means of the normal and cancer samples, and *b*-values as above. On the basis of 2000 iterations the coefficient of variation (CV) of the high and low ADC components was 2.0% and 5.9%, respectively, for the normal sample and 4.2% and 12.9% for the cancer sample. The CV of the signal fraction was 1.7% for the normal sample and 4.3% for the cancer sample. The means of the fit coefficients differed from the model values by less than 3.8%.

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High spatial resolution DWI

Diffusion weighted images of the normal and cancer tissue samples are shown in Fig. 1A. The normal tissue sample shows typical healthy gland structure with low diffusivity in the epithelium-containing voxels, intermediate diffusivity in the stroma, and high diffusivity in PBS-filled ducts. The Gleason pattern 3+4 cancer sample has no normal glands and an almost complete absence of

ductal spaces. These characteristics are consistent with earlier diffusion-weighted MR microscopy of prostate tissue (10-11).

Diffusion decay at low spatial resolution

For comparison with diffusion decay measured at low spatial resolution ~~in vivo~~in-vivo we averaged the signal obtained at each *b*-value from 10.2 mm^3 (1600 voxels) "tissue only" volumes in the normal and cancer tissue samples. Biexponential fit coefficients for these signals are shown in Table 1, together with published data from multi-*b* DWI of the prostate ~~in vivo~~in-vivo (2). Fitted data are shown in Fig. 1B. As for the data acquired ~~in vivo~~in-vivo, the ADCs of both the fast and slow diffusing components in the formalin fixed samples were lower in cancer tissue than in normal tissue. In the fixed tissue the percentage decrease of the ADCs, and the increase of slow component signal fraction, were almost identical to that seen ~~in vivo~~in-vivo.

Biexponential fits to intermediate spatial resolution diffusion decay data

To eliminate interference due to the signal arising from the PBS medium surrounding the tissue core all subsequent analyses were performed on mESD data from the "tissue only" subvolumes of each sample. The corresponding 1600×64 = 102,400 voxels from the 40 µm isotropic ADC data were used to estimate the underlying partial volumes of epithelial tissue, stromal tissue, and ductal space (11). Histograms of the biexponential fit coefficients are shown in Fig. 2. In the large majority of normal tissue voxels the major component has intermediate ADC and the minor component has very low ADC. Of the total 1600 voxels, 1530 were comprised of low (ADC < 0.6 μm^2/ms) and intermediate (0.6 < ADC < 1.9 μm^2/ms) ADC components.

In the cancer sample the major component has two significant subcomponents with mean ADC 0.84 ± 0.20 μm^2/ms and 0.09 ± 0.03 μm^2/ms. The minor component is almost entirely comprised of a subcomponent with ADC 0.09 ± 0.05 μm^2/ms. As seen ~~in vivo~~in-vivo, the mean signal fraction of the major component ($A_1/(A_1 + A_2)$) was lower in the cancer tissue than in the normal tissue.

Estimation of compartment partial volumes in diffusion decay data

The ADC histogram of the 102,400 voxels in the "tissue only" subvolume of the normal sample (Fig. 3A) can be accurately described (R^2 = 0.999) by three Gaussian components of ADC 0.82 ± 0.17, 1.31 ± 0.39, and 2.23 ± 0.29 μm^2/ms (11). Based on this histogram, voxels with ADC less than 0.88 μm^2/ms were counted as "epithelial", voxels with ADC greater than 2.00 μm^2/ms were counted as "ductal", and the remainder as "stromal". On this basis the total "tissue only" subvolume of the normal sample comprised 16% epithelium, 80% stroma, and 4% duct. These values are inside the range previously reported for normal tissue using the same method (11).

The cancer tissue histogram (Fig. 3B) can be described (R^2 = 0.998) by two Gaussian components of ADC 0.58 ± 0.22 and 0.41 ± 0.14 μm^2/ms. Consistent with normal histopathology the cancer sample is characterized by a relatively high partial volume of epithelium. The total "tissue only" subvolume was estimated to comprise 64% epithelium, 36% stroma, and less than 0.2% duct.

In both the normal and cancer tissue samples the ADC of the low diffusivity fit component is very much lower than the ADC of the "epithelial" compartment, and the ADC of the high diffusivity fit component is significantly lower than the ADC of the "stromal" compartment.

Correlation between diffusion decay signal fraction and compartment partial volumes

For correlation of biexponential signal fractions with the estimated partial volumes of epithelium and stroma sample the signal fraction of low and intermediate ADC component was calculated from the fit data according to cutoffs defined by the ADC histograms of Fig. 3. For normal tissue low ADC was defined as < 0.5 µm^2/ms and intermediate ADC as $0.5 \leq$ ADC ≤ 2.0 µm^2/ms. For cancer tissue low ADC was defined as < 0.3 µm^2/ms and intermediate ADC as $0.3 \leq$ ADC ≤ 2.0 µm^2/ms.

On the assumption that the low ADC fit component arises primarily from the low ADC epithelial compartment, and the intermediate ADC component from stromal tissue, we plotted the signal fraction of each fit component in the 1600 normal tissue voxels versus estimated epithelial and stromal partial volumes (Fig. 4A). The plots suggests a linear relationship with the R^2 value indicating that 55-60% of the variation in signal fraction can be explained by variations in the partial volume of epithelium and stroma. However, the intercept of the regression lines indicates that the low ADC component remains present even when the estimated partial volume of epithelium is zero. Similarly, the intermediate ADC component remains present even when the estimated partial volume of stroma is zero.

In the cancer tissue sample (Fig. 4B) the correlation between biexponential signal fraction and estimated partial volumes of epithelium and stroma is much weaker than in the normal tissue sample. This is possibly a result of the loss of a well defined glandular structure and consequently more heterogeneous composition of most 160 µm voxel. From Fig. 3B it is also apparent that there was a higher probability of misclassification of individual 40 µm voxels and consequently a higher uncertainty in the partial volume estimates.

Taken together these results suggest that neither the low nor intermediate ADC fit components are unique properties of either the epithelial or stromal compartments. It does however appear that the epithelial compartment in normal glandular tissue has an inherently larger low ADC component than the stroma. The intercepts suggest that “epithelium” (or more correctly, an epithelium-containing high spatial resolution voxel) is comprised of ~60% low ADC component and ~40% intermediate ADC, while the “stroma” contains only ~20% low ADC component and ~80% intermediate ADC (The correlation for the high ADC “ductal” compartment is not shown due to very low partial volumes of ductal space).

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Comment [WSP2]: Was ist das?

It appears that the relative amounts of low and high diffusivity component are distinctly different in the two compartments. This perhaps reflects a distinctly different intracellular environment in the stromal and epithelial cells and/or a difference in the extracellular space between adjacent cells. Given the different biological functions of the stroma and epithelial layer this is entirely plausible.

In our normal tissue sample the ADC of the low ADC fit component (0.23 ± 0.07 µm^2/ms) was about three times lower than that reported ~~in vivo~~in-vivo. The ADC of the intermediate (“fast ADC”) component was about two times lower. These difference can probably be attributed to the fixation process as a similar decrease in ADC was found in a previous comparison of ~~in vivo~~in-vivo and fixed prostate tissue (15). Although prostate tissue ADC appears to decrease as a result of formalin fixation the ADCs of both our biexponential fit components are considerably higher

than those reported for the cytoplasm and nucleus of isolated (unfixed) single neurons (7). It would thus be premature to conclude that our low and intermediate ADC fit components are characteristic of subcellular structure.

Summary and Conclusions

Formalin-fixed prostate tissue appears to be a good experimental model for investigation of the biophysical basis of mESD. The magnitude of differences between fixed normal and cancer tissue studies measured at medium and high spatial resolution ~~ex vivo~~ex-vivo were very similar to the changes previously reported from low spatial resolution studies ~~in vivo~~in-vivo. Although this preliminary study is based on the analysis of one sample of normal tissue and one sample of cancer tissue these samples ~~appeared to be~~are typical of their tissue type in terms of histopathology of adjacent tissue~~,~~. The diffusion behavior is similar to that observed ~~in vivo~~in-vivo~~,~~ with corresponding structures and voxel ADC statistics ~~observed on high resolution DWI, and voxel ADC statistics~~ derived from high resolution DWI.

We demonstrated that, at least in these prostate tissue ~~samples ,~~samples, the coefficients of a biexponential fit to mESD data appear to be partially determined by the underlying volume fraction of epithelial and stromal tissue. Our study suggests that epithelial and stromal tissue contain distinctly different amounts of intermediate and low diffusivity compartments.

Imaging at very high spatial resolution, in this case ~400,000 times higher than a typical prostate DWI exam ~~in vivo~~in-vivo, has permitted investigation of earlier speculations about the biophysical basis of diffusion decay observed ~~in vivo~~in-vivo. Our results are consistent with earlier reports suggesting biexponential decay may result from subcellular water compartmentation and ~~we have shown~~ that there may be distinct ~~differences in this~~ compartmentations between the epithelial and stromal parts of glandular prostate tissue.

Figure Captions

[See separate Figures doc]

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