In-Vitro Detection of Apoptosis Using Oscillating and Pulsed Gradient Diffusion Magnetic Resonance Imaging

Sharon Portnoy[1], Nicole D. Fichtner[2], Claudia Dziegielewski[1], Martin P. Stanisz[1], and Greg J. Stanisz[1,2]
[1]*Sunnybrook Research Institute, Toronto, Ontario, Canada,* [2]*Department of Medical Biophysics, University of Toronto, Ontario, Canada*

Target Audience: Those interested in using diffusion MRI methods to probe tumour microstructure and thereby provide greater specificity in the assessment of anti-cancer therapy.

Purpose: Cellular apoptosis, a common pathway towards tumor regression, is induced by many radiotherapy and chemotherapy regimens. Imaging methods which can detect this mode of cell death may be able to assess treatment response earlier than typical tumour volume measurements. Significant alterations in cellular morphology are readily apparent with apoptosis. Diffusion MRI, with its high microstructural sensitivity, is therefore well suited to the evaluation of this process. In this study, both oscillating (OGSE) and pulsed (PGSE) gradient diffusion MRI measurements were used to probe the microstructural effects of apoptosis at a broad range of spatial scales. Diffusion data were subsequently fit to the parallel-plane model (PPM) - a simple model for restricted diffusion in tissues, which describes the data in terms of 3 parameters: d, the distance between the planes (which can be viewed as the mean distance between restricting barriers in the sample), D_{free}, the intrinsic, free diffusion coefficient of the solvent, and D_{rest}, the long-time, or "restricted" diffusion coefficient.

Methods:

Sample Preparation: This study focused on *in-vitro* cultures of acute myeloid leukemia (AML-5) cells. To induce apoptosis, half the cells were exposed to the chemotherapeutic agent cisplatin at a clinically relevant dose of 10 μg/mL[1]. 72 hours after cisplatin treatment, cell pellets were formed by centrifugation and transferred into MR-compatible glass sample tubes for imaging.

MRI: Scanning was performed on a 7T preclinical MRI system (Bruker Biospin). Temperature was monitored throughout scanning and verified to be stable at 20±0.5°C. OGSE and PGSE diffusion scans were performed with a TR/TE of 1500/70 ms. In the PGSE sequence, the diffusion gradient duration, δ, was fixed at 2 ms and the spacing between diffusion gradients, Δ, was varied to provide 3 different effective diffusion times, TD_{eff}=10, 20, and 40 ms. For the OGSE sequence, pulsed gradients were replaced with a pair of sinusoidal gradients of T=30 ms duration. OGSE acquisitions were performed with both sine (OGSE-SIN) and apodized cosine (OGSE-COS) gradient waveforms[2] at a range of frequencies from 33-600 Hz. The effective diffusion time for an OGSE acquisition is related to the period, τ, of the oscillating gradient waveform. For OGSE-SIN, TD_{eff}=3τ/8 and for OGSE-COS, TD_{eff}=τ/4[3]. The combination of OGSE and PGSE scans were thus used to probe a broad range of diffusion times (0.6ms<TD_{eff}<40ms).

Histology: After scanning, cell pellets were fixed in 10% formalin for at least 1 week before being extracted from the NMR tubes and embedded in paraffin. Four 5-μm-thick longitudinal slices through the center of the cell sample were stained: two with hematoxylin and eosin (H&E) and two with terminal deoxynucleotidyl transferase UTP nick end labelling (TUNEL). H&E staining allows for visualization of cellular and nuclear morphology and cellular density, while TUNEL stains apoptotic cells.

Data Analysis: On the acquired images, regions of interest were manually drawn in each NMR tube. The mean ROI signal was subsequently fit to the parallel-plane model. For comparison, the mean ROI signal *vs.* b-value was also fit to a mono-exponential decay function to determine the apparent diffusion coefficient (ADC) for each diffusion-time/frequency.

Parallel Plane Model: Fitting the data to the PPM is equivalent to modeling the restriction effects within the sample by a series of regularly spaced (by the restricted size, d) permeable planes[4]. The parallel-plane model (PPM) was used to fit OGSE-SIN, OGSE-COS, and PGSE diffusion data globally. Briefly, fitting data to the PPM requires calculation of $F(\omega)$, the Fourier transform of the time integral of the diffusion sensitizing gradient waveform, $g(t)$ (see Eq. [1], right). Given $F(\omega)$, the signal, S, in a diffusion experiment can be expressed by Eq. [2] (right), where S_0 is the signal in the absence of diffusion gradients and $D(\omega)$ is the diffusion spectrum, which is the Fourier transform of the spin velocity autocorrelation function[5]. The diffusion spectrum for the parallel plane model is given by Eqs. [3],[4] (right)[4],[5].

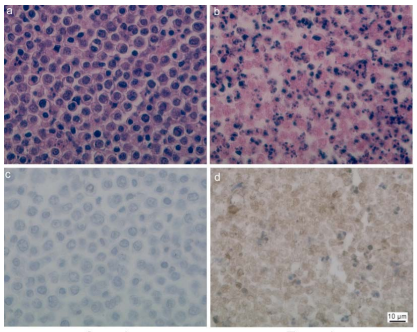

Figure 1: Representative H&E (a,b) and TUNEL (c,d) stained sections for control and treated cells. Control cells appear roughly circular in shape with nuclei that occupy most of the cell volume. For treated cells, hallmarks of apoptosis such as nuclear condensation (pyknosis) and fragmentation (karyorhexis) are visible on the H & E stained section. Under TUNEL staining, apoptotic cells appear brown and intact cellular material is counterstained blue.

PPM Equations:

$$F(\omega)=\int_0^\infty dt\,\exp(i\omega t)\int_0^t dt'\,\gamma\, g(t') \qquad [1]$$

$$S=S_0\exp\left(\frac{-1}{2\pi}\int_{-\infty}^{\infty}|F(\omega)|^2 D(\omega)\,d\omega\right) \qquad [2]$$

$$D(\omega)=D_{rest}+\sum_k B_k\frac{a_k(D_{free}-D_{rest})\omega^2}{a_k^2(D_{free}-D_{rest})+\omega^2} \qquad [3]$$

$$B_k=\frac{8d^2}{(2k-1)^4\pi^4},\ a_k=\frac{\pi^2(2k-1)^2}{d^2} \qquad [4]$$

Results: Representative H & E and TUNEL stained sections from control and treated cell pellets are shown in Fig. 1. ADC measurements are plotted in Fig. 2. The solid lines in Fig. 2 represent the PPM model fit. Average PPM parameters for control and treated cells are listed in Table 1.

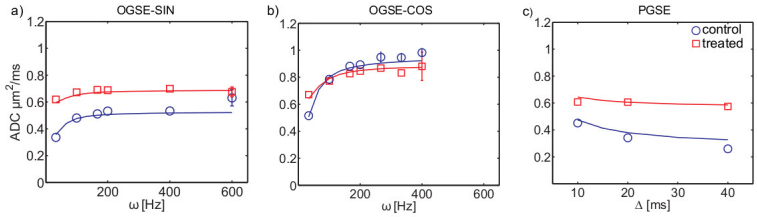

Figure 2: ADC measurements for control and treated cell pellets. Solid lines are the PPM model fit.

Discussion: Apoptotic samples exhibited significant decreases in parameters d and D_{free} and a significant increase in D_{rest}. These changes are consistent with the established morphological effects of apoptosis. In particular, the decrease in d may be a result of the combined effects of cell shrinkage, nuclear fragmentation and membrane blebbing, the decrease in D_{free} may relate to cytosolic condensation, while the increase in D_{rest} can be attributed to increased membrane permeability due to the loss of membrane integrity. By non-invasively detecting apoptosis, the methods reported in this study have the potential to improve upon current MRI methods for monitoring the response to cancer therapy. Furthermore, they may offer sufficient specificity to differentiate between apoptosis and other modes of cell death, such as oncosis or necrosis.

	Restricted size, d [μm]	D_{free} [μm²/ms]	D_{rest} [μm²/ms]
Control	5.7±0.5	1.08±0.05	0.22±0.05
Treated	3.1±0.3	0.96±0.02	0.58±0.01

Table 1: Summary of fitted PPM parameters

References:

[1] Pirnia F, *et al. Anticancer research.* 2006; 26(3A): 1765–72. **[2]** Parsons EC, Does MD, Gore JC . *Magnetic Resonance Imaging*. 2003; 21(3): 279–85. **[3]** Gore JC , *et al. NMR in biomedicine*. 2010; 23(7): 745–56. **[4]** Portnoy S , *et al. Magnetic resonance in medicine.* 2013; 69(4): 1131–45. **[5]** Stepisnik J. *Physica B*. 1993; 183: 343–50.