The Effects of Intracellular Organelles on the ADC of Water Molecules in Cells

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Introduction

MRI measurements of the apparent diffusion coefficient (ADC) of water molecules in tissue are sensitive to the effects of various pathologies, and are commonly used to characterize tissue changes in stroke and cancer. However, the underlying biophysical mechanisms influencing these variations remain poorly understood. Furthermore, the majority of MRI studies continue to assess diffusion over relatively long time scales (tens of milliseconds) such that variations in ADC reflect the integrated effects of changes in tissue structure over a broad range of spatial scales including cell density. In order to gain additional insight into the factors influencing ADC in biological tissue, we have used temporal diffusion spectroscopy—a technique capable of achieving diffusion times two orders of magnitude shorter than those previously reported—to study packed cells in vitro. This technique relies on rapid oscillations of the motion sensitizing diffusion gradient, and are typically implemented in an Oscillating Gradient Spin-Echo (OGSE) sequence. These methods were applied to a model system of packed cultured human embryonic kidney cells (293-EBNA) at gradient oscillation frequencies up to 2 kHz (effective diffusion times much below one millisecond) following manipulation/disruption of intracellular structure with drugs that alter microtubule formation (Nocodazole), actin polymerization (Cytochalasin D), and Golgi structure and function (Brefeldin A).

Methods and Results

Human embryonic kidney cells were cultured in suspension with F17 (Invitrogen, #0050092DK) supplemented with 0.1% Pluronic F68, 4 mM glutamine, and 50 mg/ml of G418. The culture was split every four days. The day of sample preparation, cell viability was measured using the trypan blue dye exclusion method. Approximately 2.5 x 10^7 cells were then transferred to 250 μL sample tubes, and N = 18 samples each were treated with one of three drugs at the following concentrations: Brefeldin A at 350 μM (Sigma-Aldrich, #B7450), Nocodazole at 100 nM (Sigma-Aldrich, #M1404), and Cytochalasin D at 5 μM (Invitrogen, #PHZ1063). Control cell samples (N = 18) received an equal volume of phosphate buffered saline (PBS) only. After 30 minutes (Brefeldin A), 60 minutes (Cytochalasin D and controls), or 90 minutes (Nocodazole) of drug exposure, cells were centrifuged at 6000 rpm for five minutes, and supernatant fluids were removed with a micropipette. Samples were then studied using a 300 MHz Varian spectrometer (Varian Inc., Palo Alto, CA) equipped with specialized gradients manufactured by Doty Scientific, Inc. (Columbia, South Carolina, USA) and maintained at 19°C for data collection. Whole sample spectroscopic data were obtained with both conventional PGSE methods using δ = 5 ms and Δ = 30 ms, and with OGSE techniques at frequencies between 200 Hz and 2000 Hz (in 200 Hz increments). Diffusion-weighted data were collected at b-values of 0, 300, and 600 s/mm², and NEX = 8.

While cell samples treated with Brefeldin A and Nocodazole showed no significant difference in ADC from control samples (p > 0.05), the ADC in Nocodazole treated cells decreased from control values at high oscillation frequencies, suggesting that the inhibition of microtubule formation may influence ADC at oscillation frequencies beyond 2 kHz. Cell samples treated with Cytochalasin D, however, showed a significant difference in ADC at the highest oscillation frequencies of 1800 Hz (p = 0.05) and 2000 Hz (p = 0.05). See Figure 1. These data suggest that the disruption of the actin/cytoskeleton network has an effect on ADC, but this effect can only be detected at very short diffusion times. Values obtained with PGSE methods were nearly identical for all treated groups and controls (p > 0.75 in all cases). Fluorescent microscopy, following the staining of cells with fluorescent markers of Golgi, microtubule, and actin structure, confirmed the morphological effects of these various treatments.

Discussion

In this study, we assessed the effects of changes in intracellular structure on tissue ADC in a model system. Disruption of the microtubule network or the Golgi complex had little effect over the diffusion times measured in this study, but disruption of the actin cytoskeleton did decrease ADC slightly at very high frequencies. No effects were detectable using long diffusion times. These studies suggest that changes within cells may be detectable using OGSE methods at high frequency, but that the integrity of major individual organelles is not a significant factor that contributes to the variations of ADC in tissues.

References


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