Diffusion and Chemical Shift of Intracellular Cesium Ions within Perfused HeLa Cells

L. Zhao[1], J. J. Ackerman[1], and J. J. Neil[1]
[1]Washington Universtiy in St. Louis, St. Louis, MO, United States

Introduction: A clear understanding of intracellular diffusion is required for proper interpretation of MR diffusion measurements performed in mammalian tissue. As a potassium analog, the MR-active cesium ion ($^{133}Cs^+$) is an excellent probe of intracellular diffusive motion. Here, the apparent diffusion coefficient (ADC) of the intracellular cesium ion was determined in a perfused HeLa cell system. The temperature dependence of the cesium ion ADC and resonance frequency were also measured.

Material and Methods: Microbeads coated with HeLa cells were packed into a 6.0-mm-ID glass tube and perfused with pre-warmed and oxygenated cesium-containing (20 mM CsCl) culture medium. After approximately 3 hours of perfusion, intracellular and extracellular cesium concentrations reached equilibrium. The apparent diffusion coefficient of intracellular cesium was estimated with a diffusion-sensitive spin-echo pulse sequence (tr = 4 ms, te = 33 ms, δ = 9 ms). Diffusion measurements at diffusion times (Δ - δ/4) of 13.5 ms and 23.5 ms were employed, corresponding to Δ of 16 ms and 26 ms respectively. Half-sine-shaped diffusion-sensitive gradients were incremented for diffusion encoding. Variable temperature studies employed a diffusion time of 13.5 ms. The diffusion coefficient of cesium ion in culture media was also measured.

Results and Discussion: Figure 1 shows diffusion-weighted spin-echo cesium-133 spectra at 13.5 ms diffusion time. The intracellular and extracellular cesium resonances are shifted by ~ 1 ppm. Diffusion signal decay was modeled as a mono-exponential function of b-value. ADC values at 37 °C are reported in Fig. 2. As expected of a compartment-sequestered ion, its ADC decreases with increased diffusion time. Figure 3 compares the temperature dependence of the intracellular cesium ADC to that of cesium in the culture media. Data were modeled as a linear function. The diffusion coefficient of cesium in the media increases by 0.04 μm^2/ms per degree Celsius while the intracellular cesium ADC increases by 0.008 μm^2/ms per degree Celsius. This difference reflects the sequestration of cesium within the HeLa cells, whereas cesium diffuses freely throughout the media. Figure 4 shows the intracellular and extracellular cesium chemical shifts. The chemical shift of the extracellular cesium shows a linear dependence on temperature, and it shifts with a rate of 0.15 ppm/°C. The chemical shift of the intracellular cesium changes at the same rate when the temperature is below 37 °C. Over a temperature range of 5 °C to 37 °C, the chemical shift difference between intracellular and extracellular cesium resonances does not change significantly. As temperature increases above 37 °C, the chemical shift difference between the two compartments grows smaller. This may be due to a heat-induced change within the intracellular environment or a marked increase in the cesium exchange rate between the two compartments.

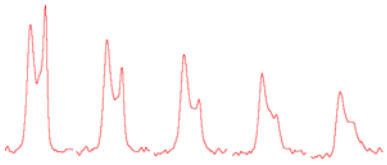

Fig. 1 Representative diffusion attenuated Cs^+ resonances acquired at b-values of 0.038, 0.22, 0.54, 1.01, 1.63 μm^2/ms. The resonance on the left arises from intracellular Cs^+ and the resonance on the right arises from extracellular Cs^+.

Conclusions: Diffusion of intracellular cesium ions sequestered within HeLa cells is approximately four times slower than that in free media. An inverse dependence of cesium ADC *vs.* diffusion time reflects restriction to displacement. Cesium chemical shift temperature dependence implies a marked change in dynamics/environment above ~ 37°C.

Acknowledgements: The authors thank Lynne Collins for her support in cell culturing. We acknowledge the funding from NIH (grant EB002083), NIH/NCI Small-Animal Imaging Resource Program (SAIRP) (grant R24 CA83060), an NCI Comprehensive Cancer Center (grant P30 CA91842) and the Green Foundation.

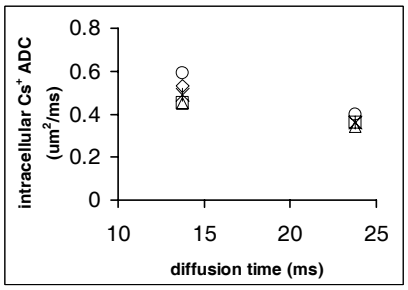

Fig. 2 Intracellular Cs^+ ADC at 13.5 ms and 23.5 ms diffusion time (37°C).

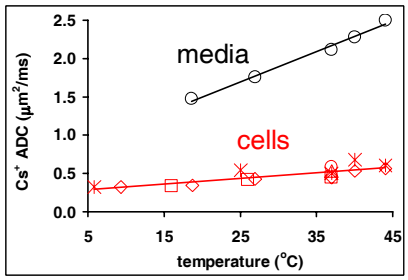

Fig. 3 Cs^+ diffusion coefficient in media and ADC in cells at different temperatures. The diffusion time is 13.5 ms.

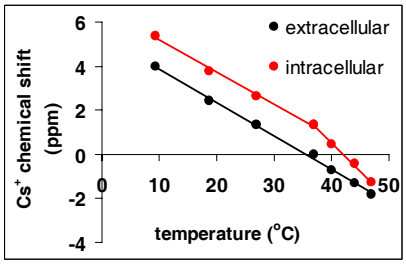

Fig. 4 Representative Intracellular and extracellular Cs^+ chemical shifts at different temperatures.