Effects of cellular mitosis on the apparent diffusion coefficient of synchronized HeLa cells

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Introduction

The apparent diffusion coefficient (ADC) of self-diffusing water molecules in biological tissue is reduced from that of a simple solution as a result of restrictive barriers. Consequently, the attenuation of the NMR signal in pulse gradient spin echo sequences (PGSE) as a function of diffusion time, in principle, provides a means of extracting information regarding the spatial scales of such restrictions. Most measurements of ADC in biological samples correspond to the regime of long diffusion intervals, when the ADC is affected by restricting boundaries of cellular dimensions or larger. Therefore, differences in ADC between tissues detected by current PGSE methods, such as changes in cellularity in tumors, reflect alterations in the number, permeability and separation of boundaries on the whole cell scale. At shorter diffusion times, the ADC is affected by restrictions that are more closely spaced and thus may reveal changes on a sub-cellular scale. We have studied the effects of cell division on ADC because it is a demonstrated cause of major changes in intracellular organization. At any time within growing tissues such as tumors, cells may be in any of several different stages of their mitotic cycle. During mitosis there is massive rearrangement of intercellular macromolecules, organelles and water shifts. Beall et al. (1) observed cyclic changes in the NMR relaxation time T1 that occur during mitosis in dividing HeLa cells, during which changes in T1 of ≈100% were seen, including large changes at times when water content was constant, implying the NMR properties reflected internal reorganization of macromolecules. ADC within the nucleus has been reported to be higher than in cytoplasm (2). We therefore chose to study synchronized populations of HeLa cells, which have a large nuclear/cytoplasm volume fraction.

Methods and Results

Cervical cancer (HeLa) cells in each of the four phases of mitotic progression (G1,S,G2,M) were prepared according to Beall, et al (1). Approximately 3 x 10^8 cells of each phase were centrifuged for 2 minutes at *750g*, then the fraction in each phase was measured using flow cytometry. The samples were then placed into a 9.4T spectrometer for data collection.

Diffusion weighted water spectra were collected from a volume within each pellet (selected from images through the sample) using a stimulated echo (STEAM) sequence, thereby allowing diffusion times in excess of the T2 of the sample. For each pellet corresponding mainly to a different mitotic phase, spectra were collected for 5 different diffusion times (12,20,40,80,150 ms) using 5 different b-values (0, 118, 465, 805, 1142 mm^2/s, with gradient cross-term contributions included). A thermal monitor confirmed a steady-state temperature within the bore equivalent to room temperature. The data were then analyzed using different models. First, the resulting diffusion data were simply fit to a monoexponentially decaying curve using least squares minimization. The resulting ADC's, as a function of both diffusion time and mitotic cell phase, for the average of the 3 most homogeneous samples of each phase (> 70% in phase) are illustrated in figure 1. In addition, because each sample in practice contained mixtures of cells in different phases, we used the data from flow cytometry as weighting coefficients in a multiple regression analysis of all the data, assuming each subpopulation contributed to the overall behavior independent of the other cell fractions. These results are shown in figure 2. Note that the flow cytometry cannot distinguish between populations of G2 and M phase cells, and therefore these phases were modeled together in the multiple regression analysis.

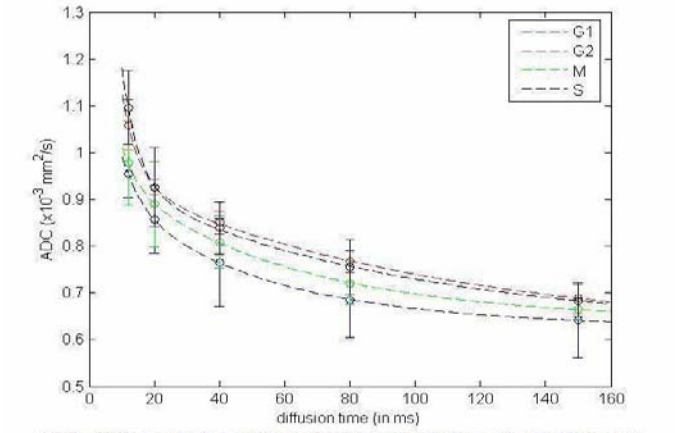

Fig 1. ADC as a function of diffusion time for cultured HeLa cells in synchronized phases of mitosis

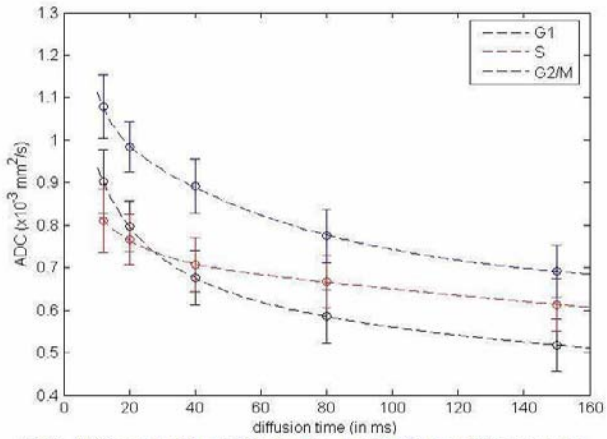

Fig 2. ADC as a function of diffusion time, resulting from multiple regression analysis of HeLa cell data

Discussion

The ADC values in the pellets were consistent with tissue values and densely packed cells. It appears that the ADC varies significantly and shows different behavior with diffusion time depending on mitotic cell phase, possibly reflecting intrinsic structural differences in the progression of the mitotic cycle. At longer diffusion times, the lowest ADC appears in the first gap (G1) phase during a time of increased RNA and protein synthesis, but appears to increase during the second gap (G2) phase, when chromatin is condensed into tightly packed chromosomes. Future studies will attempt to elucidate these variations more clearly with more sophisticated models capable of revealing other relevant biological parameters of influence, and will evaluate the influence of possible confounding factors. We also aim to measure these variations at much shorter diffusion times in an attempt to examine the spatial scales at which restrictions first play a role in self-diffusion.

References

1. P.T. Beall, et al. *Science* 192:904-907 (1976)
2. Sehy, et al. MRM 48:42-51 (2002)