Estimations of membrane permeability and the intracellular diffusion coefficient based on the pulsed-gradient spin-echo measurement and the finite difference diffusion simulation

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Introduction

The diffusion signal attenuation at high b values exhibits non-monoexponential behavior. The signal attenuation is often modeled by a biexponential function which consist of fast and slow diffusion components [1]. In some studies, the fast and slow components were attributed to extracellular space and intracellular space, respectively. However, recent studies have shown that these components do not necessarily correspond to the actual extracellular and intracellular diffusion coefficient [2]. In addition, membrane permeability makes the interpretation of diffusion attenuation more difficult. In this study, we propose a method to estimate the membrane permeability and the intracellular diffusion coefficient using the pulsed-gradient spin-echo measurement in combination with the finite difference diffusion simulation.

Materials and Methods

Leukocytes of the TCC-S cell line were used for the experiments [3]. The cells in a culture medium were centrifuged to increase the cell density, and collected into a plastic tube. The cell diameter of 17 μm and the cell density of 2.7×10^8 cells ml^{-1} resulted in the intracellular volume fraction of 68 %. Experiments were performed using a Varian 4.7 T MRI system. Diffusion signal attenuation was measured using the pulsed-gradient spin-echo method with b factors up to 6000 s mm^{-2}.

The finite difference method was used to analyze diffusion signal attenuations in cells [4]. The cell was modeled as a 15×15 μm^2 square. Volume fractions of the intracellular space and the extracellular space were set to 70 % and 30 %, respectively. Diffusion of magnetization was calculated using the following equation:

$$\mathbf{M}_{m,n}^{k+1} = s_{m-1\to m}\mathbf{M}_{m-1,n}^{k} + s_{m+1\to m}\mathbf{M}_{m+1,n}^{k} + s_{n-1\to n}\mathbf{M}_{m,n-1}^{k} + s_{n+1\to n}\mathbf{M}_{m,n+1}^{k} + \left(1 - s_{m\to m-1} - s_{m\to m+1} - s_{n\to n-1} - s_{n\to n+1}\right)\mathbf{M}_{m,n}^{k} \quad (1)$$

where $\mathbf{M}_{m,n}{}^{k}$ is the magnetization vector at the time $k\Delta t$ and at the location ($m\Delta x$, $n\Delta x$), Δx is the distance between spatial grid points, and Δt is the duration of a time step. The jump probability $s_{a\to b}$ from the point a to the point b was given by

$$s_{i-1\to i} = s_{i+1\to i} = s_{j-1\to j} = s_{j+1\to j} = s_{i\to i-1} = s_{i\to i+1} = s_{j\to j-1} = s_{j\to j+1} = D\frac{\Delta t}{\Delta x^2} \quad (2)$$

where D is the diffusion coefficient. The conditions of pulsed gradients in the simulation were identical to those in the measurement. The phase shift $\Delta\phi$ during an application of pulsed gradient (G_x, G_y) was calculated as

$$\mathbf{M}_{m,n}^{k+1} = \mathbf{M}_{m,n}^{k}\exp\left[i\gamma\left(mG_x + nG_y\right)\Delta x\Delta t\right] \quad (3)$$

The diffusion coefficients in the extracellular space and the intracellular space were set to $D_{ext} = 2.8\times10^{-3}$ mm^2 s^{-1} (the diffusion coefficient of the culture medium) and $D_{int} = d\times D_{ext}$, respectively, where d was varied from 0.0 to 1.0. The jump probability across the membrane was given by $s_{mem} = jD_{ext}\Delta t/\Delta x^2$, where j was varied from 0.0 to 1.0. The membrane permeability P was calculated from the parameter j as $P = (2jD_{int}D_{ext}/\Delta x)(jD_{ext} - jD_{int} + 2D_{int})^{-1}$.

The difference in the signal attenuation between the measurement and the simulation was evaluated by the following function:

$$F = \sum_b \left[S_{meas}(b) - S_{sim}(b)\right]^2 \quad (4)$$

where $S_{meas}(b)$ is the measured signal intensity, and $S_{sim}(b) = \Sigma_{m,n}(\mathbf{M}_{m,n}{}^{K})$ is the numerically calculated signal intensity. The membrane permeability and the intracellular diffusion coefficient were estimated from the d and j values which corresponded to a minimum F value.

Results and Discussion

Figure (a) shows the measured signal attenuation in the leukocytes. Non-monoexponential behavior was observed at b values above 1000 s mm^{-2}. The fast and slow diffusion components obtained from the conventional biexponential model were $D_{fast} = 3.3\times10^{-3}$ mm s^{-1}, and $D_{slow} = 3.2\times10^{-4}$ mm s^{-1}, respectively. Fraction of the fast component was 0.71. Figures (b)(c) show the numerically calculated signal attenuations. As observed in the measurement, the signal attenuation exhibited non-monoexponential behavior above 1000 s mm^{-2}. Both of the intracellular diffusion coefficient and the membrane permeability significantly affected the diffusion signal attenuations. An increase in the intracellular diffusion coefficient D_{int} caused a more rapid and more significant decrease in the signals. An increase in the membrane permeability caused a decrease in the signal intensity at high b values. The function F exhibited a minimum value at $j = 1.0\times10^{-3}$ and d = 0.259. These values corresponded to the membrane permeability and the intracellular diffusion coefficient of 2.8 μm s^{-1} and 7.3×10^{-4} mm s^{-1}, respectively. A clear difference was found between the slow diffusion component and the estimated value of the intracellular diffusion coefficient. In this simulation, we assumed that the diffusion coefficient and the volume fraction of the extracellular space were given values. For animal studies, these values can be measured using tracer molecules. However, for measurements of samples with unknown extracellular diffusion coefficient and volume fractions, these values can be estimated by the same approach as in the case of the intracellular diffusion coefficient and membrane permeability. Recent studies have shown that edematous states such as brain infarctions are caused by an increase in the membrane permeability. The proposed method has a potential application in non-invasive evaluation of the membrane permeability and intracellular diffusion in edemas.

References

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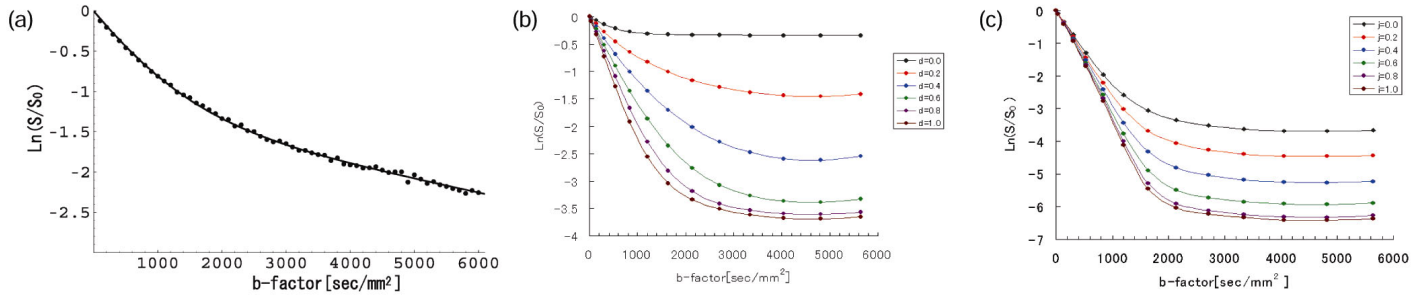

Figures: (a) Signal attenuation in leukocytes measured by the pulsed-gradient spin-echo method. (b) Simulation of diffusion signal attenuations with ratio of the intracellular diffusion coefficients to the extracellular diffusion coefficient ranging from 0.0 to 1.0. (c) Simulation of diffusion signal attenuations with membrane permeability values ranging from 0 (j = 0.0) to infinity (j = 1.0).