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Estimation of Membrane Permeability and Intracellular Diffusion Coefficients

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We propose a method for estimating membrane permeability and the intracellular diffusion coefficient using pulsed-gradient spin-echo measurement in combination with numerical simulation. The diffusion signal attenuation of leukocytes was measured with $4\pi^2q^2(\Delta - \delta/3)$ values up to 6000 s/mm^2 . For numerical simulations, the cell was modeled as a $15 \times 15 \mu\text{m}^2$ square with various membrane permeabilities and intracellular diffusion coefficients. Minimization of the difference in signal attenuations between the measurement and the simulation enabled estimations of these unknown parameters for the leukocytes. A cell sample of $2.17 \times 10^8 \text{ cells/mL}$ had a membrane permeability and an intracellular diffusion coefficient of $23 \mu\text{m/s}$ and $8.9 \times 10^{-4} \text{ mm}^2/\text{s}$, respectively.

Keywords: *membrane permeability, intracellular diffusion coefficient, pulsed-gradient spin-echo, finite-difference diffusion simulation, q-space*

Introduction

The relation between NMR signals and the diffusion of nuclear spins has been investigated in numerous studies. When a sample has no internal diffusion barriers, the relation between the diffusion coefficient D and the NMR signal intensity $S(q)$ is given by the well known equation, $S(q)/S(0) = -4\pi^2q^2D(\Delta - \delta/3)$,¹ where the parameter q is defined as $q = \gamma g \delta / 2\pi$, γ is the gyromagnetic ratio, g is the intensity of the diffusion-sensitizing gradient (q gradient), and δ and Δ are the respective pulse duration and pulse interval of the q gradient. This relation does not hold in samples that have internal diffusion barriers, such as cell membranes and porous structures. The effect of the barriers on diffusion clearly appears when the root mean square distance of diffusion $\langle x^2 \rangle = (2D\Delta)^{1/2}$ for a given gradient interval Δ is comparable to or larger than the separation between the barriers. Such barriers cause a deviation of the relation between the signal and q values from the above equation. The cell membrane is one of the diffusion barriers in biological tissues, and the separation between membranes ranges approximately from 10

μm to 50 μm . The diffusion coefficient of water at room temperature is $2 \times 10^{-9} \text{ m}^2/\text{s}$, which results in a root mean square distance of 10 μm for a q gradient interval of $\Delta = 30 \text{ ms}$. Thus, NMR signals obtained from biological tissues are clearly affected by the restriction of diffusion.²⁻⁴ In addition, biological tissues have anisotropy of diffusion because of the asymmetric structure of cell membranes.²⁻⁶ In previous studies, the relations between the NMR signal and q value have been formulated for diffusion barriers of simple geometries, such as the square, cylinder, and sphere.^{7,8} However, cell membranes have complicated geometries and finite permeabilities, and it is difficult to formulate the dependence of NMR signals on parameters such as intracellular or extracellular diffusion coefficients, volume of cells, and membrane permeability.

In the present study, we investigate the dependence of NMR signals on membrane permeability and the intracellular diffusion coefficient using finite-difference diffusion simulation. In addition, we propose a method for estimating membrane permeability and the intracellular diffusion coefficient for a sample with unknown values for these parameters.

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Principles of finite-difference diffusion simulation

NMR signals originate from magnetization (the sum of the magnetic moment per unit volume) induced in a sample by an externally applied static magnetic field. When the magnetic field is applied in the z direction, the transverse magnetization is defined by the following complex number:

$$\hat{M} = M_x + iM_y \quad (1),$$

where the real part indicates the x component and the imaginary part indicates the y component of the magnetization vector. The z component is not considered here because, under normal conditions, it does not contribute to NMR absorption signals. ^1H nuclei in biological tissues are mainly contained in water molecules. Diffusion of water in a sample causes diffusion of magnetization, which is described by the following equation in 2-dimensional space:

$$\frac{\partial \hat{M}}{\partial t} = D \left(\frac{\partial^2 \hat{M}}{\partial x^2} + \frac{\partial^2 \hat{M}}{\partial y^2} \right) \quad (2).$$

This equation is transformed into the following finite-difference equation:^{9,10}

$$\frac{\hat{M}_{m,n}^{k+1} - \hat{M}_{m,n}^k}{\Delta t} = D \left(\frac{\hat{M}_{m+1,n}^k - 2\hat{M}_{m,n}^k + \hat{M}_{m-1,n}^k}{(\Delta x)^2} + \frac{\hat{M}_{m,n+1}^k - 2\hat{M}_{m,n}^k + \hat{M}_{m,n-1}^k}{(\Delta y)^2} \right) \quad (3),$$

where k indicates the discrete time step ($t = k\Delta t$), and m and n indicate the respective spatial grid points in the x and y directions ($x = m\Delta x$; $y = n\Delta y$). When Δy equals Δx ($\Delta x = \Delta y = h$), equation (3) is simplified as follows:

$$\hat{M}_{m,n}^{k+1} = s\hat{M}_{m+1,n}^k + s\hat{M}_{m-1,n}^k + s\hat{M}_{m,n+1}^k + s\hat{M}_{m,n-1}^k + (1-4s)\hat{M}_{m,n}^k \quad (4).$$

The parameter $s = D\Delta t/h^2$ indicates the probability that a water molecule moves to adjacent grid points as a result of diffusion.

Permeability P of a membrane lying between 2 regions (region 1 and region 2) is defined as

$$J = -P(\hat{M}_{\text{mem}2} - \hat{M}_{\text{mem}1}) \quad (5),$$

where J is the diffusion flux from region 1 to region 2. As shown in Fig. 1, the parameters $\hat{M}_{\text{mem}1}$ and $\hat{M}_{\text{mem}2}$ are the magnetizations on the membrane surfaces facing region 1 and region 2, respectively. Fick's first law of diffusion gives

$$J = -D_1 \frac{\partial \hat{M}_1}{\partial n} = -D_2 \frac{\partial \hat{M}_2}{\partial n} \quad (6),$$

Fig. 1. Diffusion of magnetization across a membrane. Diffusion flux J is proportional to membrane permeability P and the difference in magnetization between the 2 sides of the membrane.

where $\partial/\partial n$ indicates differentiation in the direction perpendicular to the membrane, and the subscripts indicate the regions to which the parameters belong. When the membrane lies at the center of the 2 grid points m and m + 1 and is perpendicular to the x axis, as shown in Fig. 1, equation (6) leads to the following finite-difference equation:

$$J = -D_1 \frac{\hat{M}_{\text{mem}1} - \hat{M}_{m,n}^k}{\Delta x/2} = -D_2 \frac{\hat{M}_{m+1,n}^k - \hat{M}_{\text{mem}2}}{\Delta x/2} \quad (7).$$

The combination of equations (5) and (7) results in

$$J = - \frac{\hat{M}_{m+1,n}^k - \hat{M}_{m,n}^k}{\left(\frac{1}{2D_1} + \frac{1}{2D_2} + \frac{1}{P\Delta x} \right) \cdot \Delta x} \quad (8).$$

Equation (8) means that the effective diffusion coefficient between the grid points m and m + 1 is $1/(1/2D_1 + 1/2D_2 + 1/P\Delta x)$. The jump probability across the membrane is given by

$$s_{\text{mem}} = \frac{\Delta t}{\left(\frac{1}{2D_1} + \frac{1}{2D_2} + \frac{1}{P\Delta x} \right) \cdot (\Delta x)^2} \quad (9).$$

The magnetization immediately after the application of the excitation pulse ($k=0$) has only the x component and is identical at all grid points:

$$\hat{M}_{m,n}^0 = M_0 \quad (10),$$

where M_0 is a real number. An application of the q gradient of the intensity (G_x, G_y)^T at the position (m, n) causes a phase shift of $\gamma h \Delta t (mG_x + nG_y)$ in the magnetization. Equation (4) is modified in consideration of the phase shift as follows:

$$\hat{M}_{m,n}^{k+1} = (s\hat{M}_{m+1,n}^k + s\hat{M}_{m-1,n}^k + s\hat{M}_{m,n+1}^k + s\hat{M}_{m,n-1}^k + (1-4s)\hat{M}_{m,n}^k) e^{i\gamma h \Delta t (mG_x + nG_y)}$$

Fig. 2. Two-dimensional array of cells placed in 10 rows and 10 columns. Each cell was modeled as a $15 \times 15 \mu\text{m}^2$ square.

$$+ s\hat{M}_{m,n-1}^k + (1 - 4s)\hat{M}_{m,n}^k \times \exp[i\gamma\hbar\Delta t(mG_x + nG_y)] \quad (11).$$

MR signals are acquired at $T_E = K\Delta t$. The signal intensity is proportional to the magnetization.

$$S = \alpha \sum_{m,n} \hat{M}_{m,n}^k \quad (12),$$

where α is a constant determined from several factors, such as the ^1H density and the relaxation times.

Materials and Methods

Numerical model of cells

Simulations were performed on a 2-dimensional cell model, shown in Fig. 2. Each cell was modeled as a $15 \times 15 \mu\text{m}^2$ square and was arrayed in 10 rows and 10 columns. The separation between cells was adjusted so that the volume fraction of intracellular space was equal to that of the sample in the following experiments. The diffusion coefficient of the extracellular space, D_{ext} , was $2.8 \times 10^{-9} \text{ m}^2/\text{s}$ (the diffusion coefficient of the culture medium used in the experiments). The diffusion coefficient of the intracellular space, D_{int} , ranged from $0.0 \text{ m}^2/\text{s}$ to $2.8 \times 10^{-9} \text{ m}^2/\text{s}$, and the membrane permeability ranged from 0.0 m/s to $8.8 \times 10^{12} \text{ m/s}$. Q gradients were applied in the x direction with a pulse duration of $\delta = 25 \text{ ms}$, pulse interval of $\Delta = 50 \text{ ms}$, and inten-

sity of up to $g = 43 \text{ mT/m}$ ($q = 4.6 \times 10^4 \text{ m}^{-1}$). The separations between the grid points and time steps were $\Delta x = 1.0 \mu\text{m}$ and $\Delta t = 50 \mu\text{s}$, respectively.

Experiments

Measurements were performed using a 4.7T UNITY INOVA MR spectrometer (Varian Associates Inc., Palo Alto, California, USA). To confirm the linearity of the gradient magnetic field, the relation between the signal and q values for isopropanol ($\text{CH}_3)_2\text{CHOH}$ was obtained prior to measurements of biological cells. Q gradients were applied with a pulse duration of $\delta = 25 \text{ ms}$, pulse separation of $\Delta = 50 \text{ ms}$, and intensity of up to $g = 57 \text{ mT/m}$ ($q = 6.0 \times 10^4 \text{ m}^{-1}$). Experiments with biological cells were performed for leukocytes of the TCC-S cell line.¹¹ Because leukocytes have a spherical shape, the volume fractions of intracellular space and extracellular space in the sample can be calculated from the diameter of the cells and the number of cells per unit volume. The cells were cultivated in a CO_2 -controlled chamber. The cells in the culture medium were centrifuged to increase the cell density and were collected into a plastic tube. NMR signals of the cells were obtained under the same condition as that of isopropanol. The number of cells per unit volume and their diameters were estimated based on microscopic observation of an extracted part of the sample.

Estimations of membrane permeability and the intracellular diffusion coefficient

The membrane permeability and intracellular diffusion coefficient values for the leukocyte sample were unknown, so to estimate these parameters, the results of simulation for various values of P and D_{int} were compared with experimental results. The difference between the simulation and the experiment was evaluated using the following function:

$$F = \sum_i (S_{\text{sim}}(q_i, P, D_{\text{int}}) - S_{\text{exp}}(q_i))^2 \quad (13),$$

where $S_{\text{sim}}(q_i, P, D_{\text{int}})$ and $S_{\text{exp}}(q_i)$ are the signal intensities obtained from the simulation and the experiment, respectively. The combination of P and D_{int} corresponding to the minimum value of F gives the membrane permeability and the intracellular diffusion coefficient of the leukocytes.

Results and Discussion

Simulations

Figure 3(a) shows the results of simulation for several values of the intracellular diffusion coefficient. An increase in the q value caused a decrease in the signal intensity. The attenuation curve exhibited a clear nonlinearity at $4\pi^2 q^2 (\Delta - \delta/3)$ values

Fig. 3. Numerical simulations of the pulsed-gradient spin-echo signals of the cell model. (a) The ratio of the intracellular diffusion coefficient to the extracellular diffusion coefficient ($d = D_{\text{int}}/D_{\text{ext}}$) ranged from 0.0 to 1.0. (b) The ratio of the transmembrane jump probability to the extracellular jump probability ($j = s_{\text{mem}}/s_{\text{ext}}$) ranged from 0.0 to 1.0.

higher than 1000 s/mm^2 . The ratio of the intracellular diffusion coefficient to the extracellular diffusion coefficient, $d = D_{\text{int}}/D_{\text{ext}}$, varied between 0.0 and 1.0, with a membrane permeability of $P = 0.0 \text{ m/s}$. The attenuation curves for the parameters of $d = 0.0$ and $d = 1.0$ were not linear, though the simulation models for these parameters consisted of one diffusion component. These attenuations were attributable to a restricting effect of diffusion caused by the cell membranes. The relation $S(q)/S(0) = -4\pi^2 q^2 D(\Delta - \delta/3)$ does not hold in the restricted diffusion, and such diffusion does not result in linear signal attenuation. Figure 3(b) shows the results of simulation for several values of membrane permeability. The ratio of the transmembrane jump probability to the extracellular jump probability, $j = s_{\text{mem}}/s_{\text{ext}}$, varied between 0.0 and 1.0 ($s_{\text{ext}} = D_{\text{ext}}\Delta t/h^2$). The intracellular diffusion coefficient was equal to the extracellular diffusion coefficient. Both the intracellular diffusion coefficient

Fig. 4. Pulsed-gradient spin-echo measurement of isopropanol. The relation between the logarithm of the signal intensity and $4\pi^2 q^2(\Delta - \delta/3)$ was linear.

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 q values. Under the parameter of $j = 1.0$, the simulation model had one diffusion coefficient and no diffusion barriers. Such diffusion theoretically results in a linear signal attenuation. The lack of linearity in the attenuation for $j = 1.0$ in Fig. 3(b) was caused by errors in numerical simulations. The errors originated from the digitization of diffusion equation (i.e., the approximation of Equation [2] into Equation [3]), and a restricting effect of diffusion from the edges of the diffusion model.

Experiments

Figure 4 shows the result of pulsed-gradient spin-echo measurement of isopropanol. The logarithm of the signal intensity linearly decreased with an increase in $4\pi^2 q^2(\Delta - \delta/3)$. This result demonstrates that the gradient hardware holds for linearity up to 6000 s/mm^2 . The slope of the curve indicated that the diffusion coefficient of isopropanol is $4.3 \times 10^{-10} \text{ m}^2/\text{s}$, which is comparable to the value obtained from a previous study ($4.4 \times 10^{-10} \text{ m}^2/\text{s}$).¹² Figure 5 shows the result of pulsed-gradient spin-echo measurement of the leukocyte. We used 2 samples with cell densities of $2.17 \times 10^8 \text{ cells/mL}$ and $0.70 \times 10^8 \text{ cells/mL}$, corresponding to the respective volume fractions of the intracellular space of 55% and 18%. The diameter of the cells was $17 \mu\text{m}$. A linear relation was not observed between $4\pi^2 q^2(\Delta - \delta/3)$ and the logarithm of the signal intensity. The lack of linearity reflects the fact that the sample consists of multiple components with different diffusion coefficients and that the diffusion in the intracellular space is restricted by the cell mem-

Fig. 5. Pulsed-gradient spin-echo measurement of leukocytes. The relation between the logarithm of the signal intensity and $4\pi^2q^2(\Delta - \delta/3)$ was not linear. Samples with high cell density (2.17×10^8 cells/mL) exhibited slow signal attenuation compared to samples with low cell density (0.70×10^8 cells/mL).

Fig. 6. Experimentally obtained signals of leukocytes and the result of simulation under the condition that gives the minimum value of the performance function F (equation [13]).

brane. Samples with high cell density (2.17×10^8 cells/mL) exhibited slow signal attenuation compared to those with low cell density (0.70×10^8 cells/mL).

Estimations of membrane permeability and the intracellular diffusion coefficient

The function F gave a minimum value with $d = 0.32$ and $j = 8.0 \times 10^{-3}$ for the sample of 2.17×10^8 cells/mL. These values resulted in an intracellular diffusion coefficient and membrane permeability of 8.9×10^{-4} mm²/s and $23 \mu\text{m/s}$, respectively. Figure 6 shows the result of simulation under these conditions and the result of the experiment. The sample of 0.70×10^8 cells/mL had an intracellular diffusion coefficient of 10.3×10^{-4} mm²/s and membrane permeability of $20 \mu\text{m/s}$. The membrane per-

meability of biological cells ranges approximately from $1 \mu\text{m/s}$ to $100 \mu\text{m/s}$. Rat hepatocyte and xenopus oocyte have membrane permeabilities of $20 \mu\text{m/s}$ and $2.7 \mu\text{m/s}$, respectively.^{13,14} The membrane permeability of human leukocyte has not been measured as far as we know. Our estimated value of the membrane permeability is comparable to the values in References 13 and 14. The proposed method for estimating the diffusion parameters may possibly be affected by several factors, such as disagreement in geometry between the cell model and the actual cell, restriction of diffusion caused by subcellular structures, and computational errors in the finite-difference simulation.

In this study, 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 an unknown extracellular diffusion coefficient and volume fraction, 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. Our proposed method has potential application to non-invasive evaluation of the membrane permeability and intracellular diffusion in edemas.

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