Anisotropy of Water Diffusion in the Myocardium of the Rat

Leoncio Garrido, Van J. Wedeen, Kenneth K. Kwong, Upshur M. Spencer, Howard L. Kantor

Abstract Pulsed field gradient nuclear magnetic resonance methods combined with nuclear magnetic resonance imaging were used to determine the water diffusion anisotropy in perfused rat hearts at 37°C. It was found that the observed diffusion coefficient $D^\text{app}$ (apparent diffusion coefficient) depends on the orientation of the applied gradient $g$. When $g$ is parallel to the epicardial surface, the observed diffusivity is $D^\text{app} = 1.8 \pm 0.4 \times 10^{-9}$ m$^2$·s$^{-1}$, whereas when $g$ is perpendicular to it, diffusivity is $D^\text{app} = 2.5 \pm 0.5 \times 10^{-9}$ m$^2$·s$^{-1}$. To better characterize this directional dependence, images of the second-order diffusion tensor $D$ of the myocardium were obtained. These data demonstrate several essential features of cardiac myoarchitecture, including the helicity of fiber orientation with respect to the ventricular axis and the variation of fiber pitch angle with transmural depth. Diffusion anisotropy may be quantified in a coordinate-independent manner by the eigenvalues of the diffusion tensor. In the myocardial midwall, these eigenvalues were $E_1 = 3.29 \pm 0.57$, $E_2 = 2.01 \pm 0.42$, and $E_3 = 0.77 \pm 0.58 \times 10^{-9}$ m$^2$·s$^{-1}$ (mean ± SD). These data suggest that myocardial water diffusion is essentially unrestricted parallel to the myofilbers. They further show that failure to measure the complete diffusion tensor may lead to substantial underestimates of diffusion anisotropy in the myocardium.

Key Words • pulsed field gradient nuclear magnetic resonance • myocardium • anisotropy • diffusion tensor

Myocardial function depends not only on chamber size and inotropic stimulus but also on fiber architecture. Myocardial fibers are organized in a spiral arrangement, with subepicardial and subendocardial fibers running predominantly parallel to the long axis of the cavity and midmyocardial fibers arranged for the most part circumferentially. Fiber orientation changes can alter wall stress as well as the timing pattern and function in systole and diastole. Typically, we learn about the direction of the fibers in a myocardial segment by observing the motion of markers at various myocardial depths or by dissection after chemical fixation. These procedures can disrupt the architecture and may introduce uncertainties in the structural assessment.

The diffusion of a fluid results from random translational motions. Thus, molecular diffusion measurements can be used to probe the microstructure of the diffusant environment, with the goal of demonstrating a reduction in the diffusion coefficient as a result of encountering cell membrane barriers. Among the various techniques available to study fluid molecular displacements, the pulse field gradient (PFG) nuclear magnetic resonance (NMR) provides a simple and effective means of determining the diffusion coefficient ($D$) in many systems. $^3$-$^6$ One of its principal advantages over other techniques is that the diffusion time is well defined; only the molecular motion occurring in the interval between the gradient pulses plays a role in the experiment. Additionally, PFG NMR is a noninvasive procedure that does not require the introduction of dyes or other foreign materials to the system. In this technique a magnetic field gradient labels one species of nuclei in the sample, which is then studied as a function of position along the gradient.

This work focuses on PFG NMR methods combined with NMR imaging techniques to measure the diffusion coefficient of water in perfused rat hearts as a function of relative gradient/heart orientation.$^5$-$^8$ The diffusion anisotropy in the myocardium is indicative of local structural orientation, ie, the directionality of the fibers, myofibrils, capillaries, and intracellular space.

Theory

The PFG NMR method to measure $D$, first described by Stejskal and Tanner,$^9$ consists of a pair of gradient pulses applied before and after the 180° radiofrequency (rf) pulse of a spin-echo sequence. If there are no preferential gradient directions, the observed attenuation of the echo envelope will be given by the equation

$$A(g) = A(0) \exp[-K g^2 D]$$

where $A(g)$ is the amplitude of the echo in the presence of a gradient $g$, $\rho(r_0)$ represents the a priori probability of finding a molecule at position $r_0$, $P(r_1 | r_0, \Delta)$ is the probability that a molecule initially at $r_0$ will move to $r_1$ within a time $\Delta$, $\gamma$ is the gyromagnetic ratio, and $\delta$ is the gradient pulse duration. In the case of a homogeneous and isotropic medium, and assuming that $\delta \gg \Delta$, the solution to this equation can be written as

$$A(g) = A(0) \exp[ -K g^2 D]$$

where $K = (\gamma \delta)^2 (\Delta - \delta/3)$. However, if there are barriers or other molecular arrangements within the system that lead to an orientational dependency of the translational mobility of the diffusant, the self-diffusion coefficient must be described by a tensor. In this case,

$$A(g) = A(0) \exp[ -K g \cdot D g]$$

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where $D$ is now a symmetrical second-order tensor: $D = \{D_{ij}\}$, so that for any vector $g$ the product $(g^T D g)$ is a scalar. In the isotropic case, Equation 3 reduces to Equation 2, wherein $D = D I$, where $I$ is the identity matrix.

### Materials and Methods

All studies were performed on an MSL 400 spectrometer/imager (Bruker Instruments, Billerica, Mass) equipped with an Oxford 9.4-T (proton frequency at 400.13 MHz), 8.9-cm vertical-bore superconducting magnet. $^1$H NMR diffusion-weighted images were obtained by a modified two-dimensional Fourier transform spin-echo technique with an echo time of 15.5 milliseconds. The modification to the pulse sequence consists of adding a pair of gradient pulses as shown in Fig 1. The separations ($\delta$) between the diffusion gradient pulses and their width ($\delta$) were kept constant for all experiments and equal to 2 and 5 milliseconds, respectively. Diffusion-weighted images in the direction of interest were obtained by varying the amplitude of the gradient pulses between 0 and 0.30 T·m$^{-1}$. To calculate the three-dimensional diffusion tensor, seven images were acquired, one with zero gradient and the others with gradients, $g$, of 0.14 T·m$^{-1}$ in amplitude applied in each of six directions: $(g_x, g_y, g_z) = \{1,0,0\}, (0,1,0), (0,0,1), (1/\sqrt{2}, 1/\sqrt{2}, 0), (1/\sqrt{2}, 0, 1/\sqrt{2}), (0, 1/\sqrt{2}, 1/\sqrt{2})$. The selective excitation was achieved with a 1-millisecond amplitude-modulated rf pulse. The slice thickness was 1 mm. The pulse repetition time was 0.72 seconds. The imaging time ranged from 3 to 6 minutes. The field of view was 19×22 mm, with a resolution of 128 x by 64 or 128 y-pixels, respectively.

Diffusion imaging experiments were performed on perfused heart preparations. Hearts ($n=6$) were excised from Sprague-Dawley male rats (350 to 400 g) and perfused through an aortic cannula in a 20-mm-outlet diameter NMR tube. The perfusion apparatus was similar to that described in the literature. The perfusion medium was a modified Krebs-Henseleit buffer solution. The temperature was maintained at 37°C, and the perfusate was bubbled with a gas mixture of 95% O$_2$/5% CO$_2$ to give a P$_{O_2}$>400 mm Hg. The pH of the medium was maintained between 7.35 and 7.45. A ventricular drain and a water-filled balloon to measure developed pressure were placed in the left ventricle. All hearts included in the study developed systolic pressure >90 mm Hg. Before imaging, the hearts were arrested by an increase in the KCl concentration from 4.7 to 30 mmol · L$^{-1}$ in the original medium.

Diffusion anisotropy images were constructed by solution of the matrix Equation 3 for the coefficients of the diffusion tensor $D_{ij}$ at each point, then generation of images that represent either particular directional components of $D$, eigenvalues of $D$, or the orientations of $D$ at each location by means of three-dimensional graphics.

### Results

Diffusion anisotropy may be described either in a coordinate-independent manner or in coordinates defined by cardiac anatomy. As an example, Fig 2 shows three images in a short axis of one heart acquired at the same location and with similar diffusion gradient amplitude, 0.3 T·m$^{-1}$, but different orientation. The gradients are applied along three orthogonal axes defined as $x$ (left to right), $y$ (top to bottom), and $z$ (image plane toward reader) corresponding to Fig 2a, 2b, and 2c, respectively. Panels a and b show a bipolarity of signal intensity around the ventricle, consistent with myofibrillar polarization parallel to the ventricular surface.

To calculate $D$ as a function of the relative orientation between the myocardium and $g$, a gradient was applied in the $x$ direction, and its intensity was incremented through from 0.07 to 0.30 T·m$^{-1}$ in four linear steps. The $D$ values were established by linear least-squares of $\ln[\Lambda(g)]$ versus $b$ ($=K_g^2$), the natural log of Equation 2. Diffusion measurements were made for regions of interest, myocardial sectors of $10^2$ in circumferential extent, within which the mean orientation of the epicardial contour was either parallel or perpendicular.
Fig 3. Graph showing variation of the nuclear magnetic resonance signal intensity as a function of the diffusion gradient (x direction) amplitude in regions \( \parallel (c) \) and \( \perp (\lambda) \).

ular to the diffusion-sensitizing x gradient. Since the \( D \) measurements with a single gradient can give only an estimate of the true value, the resulting apparent diffusion coefficients (\( D^{\text{app}} \)) are denoted \( D^{\text{app} \parallel} \) and \( D^{\text{app} \perp} \), since the gradient orientation is parallel or perpendicular, respectively, to the epicardial mean orientation of the region. One such fit is shown in Fig 3, and the measurements of \( D^{\text{app} \parallel} \) and \( D^{\text{app} \perp} \) for a series of hearts are summarized in the Table.

A coordinate-independent characterization of diffusion anisotropy is provided by the eigenvalues of the diffusion anisotropy tensor and their ratios. Rendered images of the three-dimensional diffusion tensor of the myocardium are shown in Fig 4. In Fig 4A, an octahedron has been constructed at each voxel, the axes of which span the three eigencomponents of the diffusion tensor \( D \) at each location. Fig 4B shows these data as a three-dimensional stereo pair of images in which the largest eigencomponent of \( D \) at each voxel is represented by a line segment of appropriate length and orientation; this component indicates the direction of maximal water mobility. In this cross section of the heart, diffusion anisotropy shows an overall helicity of orientation counterclockwise toward the base of the heart (perpendicular to the image plane, toward +z). The diffusion also reveals a "patchy" appearance, with moderate linear discontinuities of diffusion evident within the myocardium, perhaps indicative of groupings of myofibrils into macroscopic bundles. Increased directional heterogeneity is seen near the insertions of the right ventricular free wall into the interventricular septum where the septal and right ventricular fibers become confluent.

The eigenvalues of the diffusion tensor provide coordinate-independent measures of diffusion. For the study shown in Fig 4, the mean values of the eigenvalues of diffusion were measured for an annular region of interest covering the myocardial midwall (25% to 75% of wall thickness). In units of \( 10^{-9} \text{ m}^2 \cdot \text{s}^{-1} \), mean\( \pm \)SD, these were found to be

\[
\begin{align*}
(4a) \quad & E_1 = 3.29 \pm 0.57 \\
(4b) \quad & E_2 = 2.01 \pm 0.42 \\
(4c) \quad & E_3 = 0.77 \pm 0.58
\end{align*}
\]

The ratio of the means of greatest and least diffusivities, \( E_1/E_3 = 4.2 \), represents a coordinate-independent index of total anisotropy. Note that as \( E_2 = E_3 \), it follows that two crossfiber directions are inequivalent.

**Discussion**

Measurements of water diffusion in the myocardium by means of NMR imaging demonstrate profound directional anisotropy. The values observed for the diffusion coefficients are higher in zones where \( g \) is parallel to the epicardial surface and lower where \( g \) is perpendicular. The results of a previous study\(^{11} \) performed on excised heart tissue from rats at room temperature show a significant reduction in \( D^{\text{app}} \) of tissue water compared with that of free water, \( D^{\text{app} \parallel} / D_{\text{water}} = 0.36 \). This ratio is smaller than those obtained in this study of 0.60 and 0.83 for \( D^{\text{app} \parallel} / D_{\text{water}} \) and \( D^{\text{app} \perp} / D_{\text{water}} \) respectively. The discrepancy between these results might be due to differences in the experimental conditions. In Reference 11, the measurement is performed in a nonliving sample of the left ventricular apex, in which fiber orientation may be expected to change rapidly in a small volume, reducing the observed anisotropy through a partial-volume effect.

Also, it should be mentioned that in the diffusion-imaging experiments, the interaction between the imaging gradients and the diffusion gradients leads to the appearance of additional terms (ie, crossterms)\(^{9} \) in Equations 2 and 3 that have not been included in our calculations. The errors introduced in the evaluation of \( D^{\text{app}} \) by neglecting those terms were estimated to be <5% of the calculated values.

Our results demonstrate that accurate quantification of diffusional anisotropy requires an imaging approach that characterizes the diffusion tensor fully in three dimensions. It is clear that particular directional measurements of diffusion will reflect the true anisotropy only if these directions coincide with the eigenvectors of the diffusion tensor corresponding to the greatest and least eigenvalues throughout a region of interest. Methods that measure \( D^{\text{app}} \) in orthogonal coordinates may substantially underestimate diffusion anisotropy even when based on cardiac coordinates, as for example \( D^{\text{app} \parallel} / D^{\text{app} \perp} = 1.4 \), which is only one third of the observed \( E_1/E_3 = 4.2 \).

We should point out that myocardial diffusion appears essentially unrestricted in its direction of greatest mobility compared with free water. Previous studies have found that cell membranes do not have a significant effect on water diffusivity for \( \Delta \) between 2.2 and 60 milliseconds.\(^{11} \) Studies on skeletal muscle show similar results, suggesting that mainly the intracellular structures are responsible for the restriction observed.\(^{12,13} \)

Considering that the mean-square displacement of a
free molecule in a particular direction \( \langle x^2 \rangle^{1/2} \) is given by the Einstein relation

\[
\langle x^2 \rangle = (2D\Delta)^{1/2}
\]

and that \( D \) of free water is \( 3 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1} \) at 37°C,\(^\text{14}\) under the experimental conditions of this study (\( \Delta = 5.3 \) milliseconds), we have \( \langle x^2 \rangle = 6 \mu \text{m} \). Given that myocardial cells are typically 10 to 15 \( \mu \text{m} \) in diameter and at least threefold longer,\(^\text{15}\) it is reasonable to conclude that the chief hindrance to translational molecular motion results from interactions between water and the cellular cytoplasm (i.e., proteins, myofibrils, etc).

Conclusions

NMR imaging has the capacity to map the three-dimensional tensor of water diffusion in intact living tissues. In the rat myocardium, water diffusivity is found to have significant anisotropy. It follows that accurate measurement of water diffusivity in the myocardium requires assessment of the full diffusion tensor, which requires, in the absence of a priori knowledge, at least six independent directional measurements of diffusion plus one null measurement. The direction of maximal diffusivity, moreover, correlates with the expected myofibril orientation on a regional basis. Although substantial crossfiber (\( E_\perp \)) anisotropy is observed, its ultrastructural significance is not yet clear. Fiber orientation is a key determinant of the constitutive properties of the myocardium, both active and passive. Thus, nondestructive imaging of diffusion anisotropy using NMR promises to provide a new window on the structure of the intact heart.

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