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Assessment of the Effects of Cellular Tissue Properties on ADC Measurements by Numerical Simulation of Water Diffusion

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Abstract

The apparent diffusion coefficient (ADC), as measured by diffusion-weighted MRI has proven useful in the diagnosis and evaluation of ischemic stroke. The ADC of tissue water is reduced by 30-50% following ischemia and provides excellent contrast between normal and affected tissue. Despite its clinical utility, there is no consensus on the biophysical mechanism underlying the reduction in ADC. In this work, a numerical simulation of water diffusion is used to predict the effects of cellular tissue properties on experimentally measured ADC. The model shows that the biophysical mechanisms responsible for changes in ADC post-ischemia depend upon the time over which diffusion is measured. At short diffusion times, the ADC is dependent upon the intrinsic diffusivity, while at longer, clinically relevant diffusion times, the ADC is highly dependent upon the cell volume fraction. The model also predicts that at clinically relevant diffusion times, the 30-50% drop in ADC after ischemia can be accounted for by cell swelling alone when intracellular T₂ is allowed to be shorter than extracellular T₂.

Keywords

Diffusion; apparent diffusion coefficient; numerical simulation; ischemia

Introduction

The idea to use NMR to measure diffusion was introduced by Torrey in 1956 [1]. The clinical utility of diffusion-weighted MRI (DWMRI) was realized in the early 1990s in the evaluation of ischemic stroke. Within minutes of onset, DWMRI exhibits hyper-intensity in regions of the brain affected by acute stroke, while T₂-weighted images remain unaffected. The apparent diffusion coefficient (ADC), a quantitative measure of the diffusion of water in tissue, decreases 30-50% in ischemic regions of the brain [2,3]. While these results have had significant clinical utility, there remains no consensus on the biophysical mechanisms causing the drop in ADC. Several mechanisms have been proposed, including increases in the intracellular volume fraction (IVF) [3], increased tortuosity of extracellular spaces [4], increased membrane permeability [4] and decreases in the diffusion of water in the intracellular space [5,6]. Because a large number of tissue parameters have been

hypothesized to affect the ADC, mathematical models of water diffusion are useful to assess the role of each parameter on the ADC.

In free diffusion, the signal from DWMRI decays exponentially with increasing b-value, and is characterized by the intrinsic diffusion coefficient, D . However, the diffusion of water in tissue is not free: water interacts with lipid membranes, macromolecules, and other cellular and extracellular contents causing the signal decay to deviate from the monoexponential decay observed in free diffusion [7,8]. The ADC lumps all of these interactions into a single ‘apparent’ diffusion coefficient, which is calculated by fitting the DWMRI signal to an exponential decay over a specific range of b-values, typically between 0 and 1000 s/mm². Therefore, the ADC provides little indication of the specific biophysical mechanisms contributing the DWMRI signal decay. Other analyses of diffusion fit the non-monoexponential signal decay to bi- or triexponential decays. It was originally hypothesized that each component of the signal decay described a tissue compartment. However, the calculated volume fractions of intracellular and extracellular compartments from multi-component analysis did not match physiologic volume fractions measured by histology [7].

Several researchers have developed mathematical models to describe diffusive signal decay explicitly in terms of physical tissue properties. Tanner *et al.*, using a short gradient pulse (SGP) approximation, found an analytical expression for signal decay due to diffusion between two reflective parallel plates [9]. Other models of restricted diffusion have been published using the Gaussian phase distribution approximation to find signal decays from diffusion restricted between parallel plates or within a sphere [10,11]. These analytic models have been used to represent the diffusion of water inside cells restricted by the lipid membrane, but do not account for water in an extracellular compartment or exchange between these compartments. Stanisiz *et al.* generalized the Tanner *et al.* model by solving a set of differential equations to describe exchange between compartmentalized restricted intracellular and tortuous extracellular water [12]. Vestergaard-Poulsen *et al.* extended the Stanisiz *et al.* model to incorporate compartmental dependent T₂ decay and the effect of a finite echo time, TE [13]. In addition to these compartmental models, several computational models of diffusion have been developed. These models utilize a finite element (FE) or finite difference (FD) approximation to solve simplified Bloch-Torrey equations on an explicitly defined geometry [14,15]. For example, Chin *et al.* used a FD approximation to investigate the effect of myelin on diffusion restricted in axons [16]. Using the SGP approximation, it is possible to utilize a Fourier transform relationship between the diffusive signal decay and the effective diffusion propagator (EP), a volume averaged diffusion profile [17]. This formalism has been used in both in computational models as well as experiments and constitutes the theoretical basis of q-space-imaging.

Despite the large number of models available, no single model has described all aspects of the diffusion-weighted MR signal and the changes seen in the ADC of tissue water following ischemia. Given the large number of biophysical parameters with the potential to influence the ADC, it is useful to assess the effect of changes in each parameter on the ADC. The goal of this paper is to evaluate cellular and experimental MR parameters that have a significant influence on DWMRI results. A 3D finite difference model of water diffusion is presented, which incorporates T₂ decay into the EP formalism. The model calculates diffusion within a geometry of regularly spaced cubic cells taking into account the experimental parameters of diffusion time (Δ), and TE as well as tissue parameters, including intracellular volume fraction (IVF), cell size (L_{cell}), membrane permeability (P_{mem}), intracellular and extracellular diffusion coefficients (D_{int} and D_{ext}), and intracellular and extracellular T₂ coefficients (T_{2_{int}} and T_{2_{ext}}).

Methods

The diffusion equation describing MR signal, including T2 decay is

$$\frac{dC}{dt} = \nabla \cdot (D \nabla C) - \frac{C}{T_2} \quad [1]$$

where C is the concentration of signal from water, D is the free diffusion coefficient, and T_2 is the T2 relaxation rate. In the current model, tissue is represented as an array of cubic cells arranged at regular intervals with an intracellular volume fraction, $IVF = (L_{\text{cell}}/L_{\text{sep}})^3$ (Fig. 1a). Eqn. [1] was solved using an explicit forward-time center-spaced finite difference method on a regular mesh of grid points (Fig. 1b). The domain is extended in the diffusion-weighting direction such that an insignificant amount of water reaches the ends of the domain at the longest diffusion time, Δ . The array of cells is assumed to be periodic in the directions perpendicular to diffusion-weighting. By symmetry, zero-flux boundary conditions apply on the sides of the domain. Unless otherwise noted, simulations were run with parameters $D_{\text{int}} = 1.0 \mu\text{m}^2/\text{ms}$ [12,13], $D_{\text{ext}} = 3.0 \mu\text{m}^2/\text{ms}$, $T_{2\text{ext}} = 150 \text{ ms}$, $IVF = 80\%$ [18,19], $L_{\text{cell}} = 10 \mu\text{m}$ [20], $P_{\text{mem}} = 0.001 \text{ cm/s}$ [12,13,21,22], $\Delta = 50 \text{ ms}$, and $TE = 80 \text{ ms}$. The value of D_{ext} was set to the free diffusion coefficient of water at body temperature [23]. The value of $T_{2\text{ext}}$ was set between values measured in gray matter and CSF [24]. However, simulations showed that ADCs were insensitive to $T_{2\text{ext}}$ in a range of 100 to 250 ms. The effect of values and changes in the other modeling parameters is the subject of the paper and are described in the Results and Discussion sections below. The intracellular exchange rate is given by $k_i = P_{\text{mem}} \cdot SA/V = P_{\text{mem}} \cdot 6/L_{\text{cell}}$, where SA/V is the surface area to volume ratio. The model was implemented in C++ with Message Passing Interface (MPI) and run on a Linux cluster.

T2 Relaxation

As shown in Fig. 2, the time domain in the simulation is divided into three regimes based upon the type of signal decay occurring. In all three regimes, the signal from water decays due to T2 relaxation. However, signal decay also takes place because of diffusion between the two gradients. Since the motion and decay of water in each regime is described by Eqn. [1], concentration distributions are calculated with a finite difference approximation to Eqn. [1] for each regime, and include the effect of T2 decay. The simulation calculates an EP for the regime between the diffusion gradients, while weighting each diffusion distribution to match initial and final conditions between the three regimes. If T2 is uniform across the model tissue, the weights in the EP are also uniform. However, if there are compartmental differences in T2, the weights vary between compartments, causing a longer lived compartment to be more heavily weighted in the composite signal decay. Exchange of water across the cell membrane causes weights to vary spatially within the two compartments.

The Effective Propagator (EP)

To calculate water diffusion within the arena of cells, a series of FD diffusion simulations are carried out, in which the initial condition is a unit concentration at an individual grid point, i.e. $C(x | x_0, t = 0) = \delta(x - x_0)$. Given the symmetry of the geometry, only a few grid points in and around the central cell have to be considered. During diffusion weighting, the molecular motion of water in the direction of the diffusion gradient causes a phase shift, $\Delta\phi$, proportional to the translational displacement, given by $\Delta\phi = \gamma G \delta (x - x_0)$, where γ is the gyromagnetic ratio, G is the gradient pulse magnitude, and δ is the width of the gradient pulse. The signal decay S at a particular diffusion time, Δ , is then calculated by taking the expectation on the phase, $\langle e^{i\phi} \rangle$. In 1D, for simplicity, this is expressed as

$$S_{\Delta}(q=\gamma G\delta) = \iint C(x, \Delta) e^{-iq(x-x_0)} dx dx_0 \quad [2]$$

The inner integral is the expectation on the phase of the diffusion distribution, while the outer integral averages over all starting locations. Changing the order of integration and introducing a change of variables gives

$$S_{\Delta}(q) = \int \langle C(x+x_0, \Delta) \rangle e^{-iqx} dx \quad [3]$$

and the EP is

$$\langle C(x+x_0, \Delta) \rangle = \int C(x+x_0, \Delta) dx_0 \quad [4]$$

which is a measure of the average water displacement.

T2 decay between the two diffusion gradients is accounted for by including T2 in Eqn [1]. To incorporate T2 decay outside the diffusion gradients into the model, diffusion distributions are weighted to match final and initial conditions between the three regimes in the simulation. The EP is modified to

$$\langle C(x+x_0, \Delta) \rangle = \int w_1(x_0) C(x+x_0, \Delta) w_2(x+x_0) dx_0 \quad [5]$$

where w_1 and w_2 are weights for the T2-decay regimes before and after the diffusion gradients, respectively:

$$w_1(x) = \int C(x|x_1, \tau) dx_1 \quad [6]$$

$$w_2(x_2) = \int C(x|x_2, \tau) dx \quad [7]$$

The variables x_1 and x_2 represent the starting locations for water distributions in the T2-weighting regimes.

A change of variables, $b = q^2\Delta$, allows the signal decay to be fit to an exponential decay, $S = S_0 e^{-bADC}$ for b values between 0 and 1000 s/mm², which is consistent with clinical convention. While the simulated decay curves are not monoexponential over a large range of b -values, they are adequately fit to a single exponential decay over this small range.

Intracellular Water

When exchange occurs between the intracellular and extracellular compartments, water can experience both compartments over the course of the experiment, blurring the distinction between intracellular and extracellular water. An apparent intracellular EP can be produced by considering only water residing within the intracellular compartment at the time of data acquisition. From the compartment specific EP, an apparent intracellular signal decay can be calculated as described in the previous section.

Results

Simulated signal (total signal and intracellular signal) is plotted vs. b-value in Fig. 3, for values of membrane permeability corresponding to a range from free diffusion (completely permeable) to completely impermeable. The analytical solution to signal decay in free diffusion is a monoexponential decay, which is represented in Fig. 3 as a solid line, assuming a diffusion coefficient of $D = 3.0 \mu\text{m}^2/\text{ms}$. The signal decay of the intracellular compartment calculated by the FD model agrees with the Tanner *et al.* model (dotted line) for the case of impermeable membranes.

Effect of permeability on ADC

ADC values calculated from fitting decay curves between 0-1000 s/m² are plotted as a function of membrane permeability in Fig. 4 at different values of D_{int} (3.0, 1.0 $\mu\text{m}^2/\text{ms}$), $T_{2\text{int}}$ (150, 50, 25 ms) and Δ (10 – 70 ms). The shaded area represents physiologically relevant values of membrane permeability. In general, the ADC decreases as the cell membrane becomes less permeable, and this effect is more pronounced at longer diffusion times. With large differences between $T_{2\text{int}}$ and $T_{2\text{ext}}$, the ADC increases as the membrane becomes impermeable. This is a combined effect of T2 filtering and exchange. As the membrane restricts the exchange of water between the two compartments, a shorter T2 in the intracellular compartment decreases the influence of the restricted intracellular compartment on the ADC. Changes in D_{int} have a significant effect on the ADC when the membrane is permeable or at short diffusion times, but D_{int} has little effect on the ADC at low P_{mem} and longer diffusion times.

The physiologic values of membrane permeability of water diffusion have been measured for red blood cells at a value of $P_{\text{mem}} = 0.003 \text{ cm/s}$, which are considered more permeable than other cell lines [21,22]. Stanisiz *et al.* [12] and Vestergaard-Poulsen *et al.* [13] both fit to membrane permeability in the range of 0.0006 cm/s to 0.0019 cm/s. In the results presented below, a value of $P_{\text{mem}} = 0.001 \text{ cm/s}$ was used.

Effect of intracellular diffusivity on ADC

ADC is plotted vs. intracellular diffusivity, D_{int} , in Fig. 5 at different Δ (10 – 70 ms) and $T_{2\text{int}}$ (150, 50 and 25 ms). Increases in D_{int} at short diffusion times tend to increase the ADC while at long diffusion times D_{int} has relatively little effect on ADC. At short diffusion times, a significant fraction of the water has not experienced the restrictive membrane and the ADC is sensitive to D_{int} . However, at longer diffusion times much of the water has already experienced the cell membrane, making the ADC relatively independent of D_{int} . This effect is independent of $T_{2\text{int}}$, although ADCs are higher with decreased $T_{2\text{int}}$ due to T2 filtering.

Effect of T2 heterogeneity on ADC

The effect of intracellular T2 on the ADC is shown in Fig. 6 at different diffusion times and values of D_{int} . At $T_{2\text{int}} = 150\text{ms}$, T2 is homogeneous in the model. In general, a decrease in $T_{2\text{int}}$ reduces the influence of restricted intracellular water on the signal decay, and increases the calculated ADC. The three panels represent simulations run at different values of D_{int} , and shows that the ADC is relatively independent of diffusion time at longer diffusion times or at low D_{int} .

Effect of intracellular volume fraction on ADC

The ADC has a strong inverse correlation with IVF, as shown in Fig. 7. The IVF is increased by swelling the cells, increasing the volume of the intracellular space at the expense of the extracellular space. Over the range of IVF investigated (0.63 – 0.90), the

ADC decreases with increasing IVF, and the decrease is sensitive to differences in T_2 between the intracellular and extracellular spaces. As $T_{2\text{int}}$ decreases, not only does the ADC increase, but the decrease in ADC with IVF also increases.

Effect of TE on ADC

By allowing a compartmental difference in T_2 , a dependence of the ADC on TE could be predicted. The effect of TE on the ADC is shown in Fig. 8 at a diffusion time, $\Delta = 60$ ms. T_2 filtering is observed as an increase in the ADC with TE due to differences in T_2 . When $T_{2\text{int}}$ and $T_{2\text{ext}}$ are identical, there is no T_2 filtering and, as would be expected, there is no dependence of ADC on TE. At $T_{2\text{int}} = 50$ ms, one third that of $T_{2\text{ext}}$, there is a slight dependence of ADC on TE and at $T_{2\text{int}} = 25$ ms, this dependence is amplified.

Discussion

The model described in this paper reproduces results of several other previously reported models and adds new insight into the potential influences of intracellular T_2 on experimentally measured ADC. The model predicts an exponential signal decay from free diffusion, and accurately reproduces the well-known Tanner model of restricted diffusion in the case of impermeable membranes [9]. With homogenous T_2 ($T_{2\text{int}} = T_{2\text{ext}} = 150$ ms), diffusion models have predicted a decrease in ADC with restriction and an ADC that decreases with diffusion time [22], which is also predicted by the present model.

Fig. 4 plots the ADC within a complete range of membrane permeability, from free diffusion to impermeable membranes. Within the physiologic range, the change in ADC with membrane permeability depends primarily upon the T_2 difference between intracellular and extracellular compartments. With homogenous T_2 , an increase in ADC is found with membrane permeability. Decreasing $T_{2\text{int}}$ reduces the change with membrane permeability, and eventually inverts the trend. A recently published model also predicted a decrease in the ADC with exchange rate when there are large differences between $T_{2\text{int}}$ and $T_{2\text{ext}}$ [13]. The initial decrease in ADC with increasing permeability (from impermeable in the right hand column plots of Fig. 4) is a direct result of exchange affecting T_2 filtering of the intracellular and extracellular compartments. Further increases in permeability reduce the effect of membrane restriction and raise the ADC to values consistent with the fast exchange limit.

The model predicts that differences in T_2 between compartments can have a striking influence on the ADC. A faster decaying intracellular compartment weights the acquired signal toward the tortuous, yet ultimately unrestricted, extracellular compartment, causing the ADC to increase. By decreasing $T_{2\text{int}}$ relative to $T_{2\text{ext}}$ the predicted ADC is in the range measured clinically in normal tissue, between 0.7 and $1.0 \mu\text{m}^2/\text{ms}$. Such compartmental differences in T_2 have been considered in previous diffusion models [13,25]. Szafer *et al.* investigated compartmental T_2 filtering, but neglected T_2 differences after their model suggested up to a 2x difference between compartmental T_2 values had negligible effect on the ADC. More recent work has suggested larger T_2 differences between compartments [13,26,27].

T_2 filtering is mediated by the difference in the compartmental T_2 values and the intracellular exchange rate relative to the experimental timescale. The dependence upon the exchange rate is evident in Fig. 4 where the ADC increases as the membrane becomes impermeable. Given a low exchange rate, and compartmental difference in T_2 , T_2 filtering can be observed as a change in ADC with TE. Increases in ADC with TE have been previously measured, although the increase did not reach statistical significance [28]. Similarly, this model predicts an increase in the ADC with TE when there are T_2 differences between the two compartments. The increase is strongly dependent upon the magnitude of

the T2 difference, but also dependent upon the exchange rate of water between the two compartments.

Published estimates on the compartment specific values of T2 vary widely. For instance, Assaf and Cohen hypothesize $T2_{\text{ext}}$ to be faster than $T2_{\text{int}}$ after measuring an increase in the volume fraction of a slow diffusing component of water when carrying out diffusion-weighted spectroscopic experiments with TEs from 70 to 550 ms in excised bovine optic nerve suspended in a saline solution [29]. However, the tissue was not perfused during the experiments, making the measurements hard to relate to living tissue. Also, their experimental setup (TE range from 70 to 550 ms) would make it impossible to detect T2 populations in the few tens of milliseconds. In contrast to this, Buckley *et al.* predict a $T2_{\text{int}}$ as low as 20 ms after observing a slow diffusing component of water to decrease with TE in perfused hippocampal slices [27]. Vestergaard-Poulsen *et al.* also predicted a low $T2_{\text{int}}$, approximately 20 ms, by fitting experimental data obtained from gray matter to a model of diffusion in which T2 is allowed to vary within tissue compartments [13]. Silva *et al.* estimated $T2_{\text{int}}$ to be 42 ms, but this measurement was done in the presence of a susceptibility contrast agent in the extracellular space [6]. The only direct experimental measurements of $T2_{\text{int}}$ were conducted by Schoeniger *et al.*, where they measured a cytoplasmic $T2 = 34$ ms in single neurons isolated from *Aplysia californica* [26]. More experimental work is needed to directly measure values of $T2_{\text{int}}$ and $T2_{\text{ext}}$ to validate these findings and the results of this model.

It is often assumed that slow exchange in combination with compartmental differences in T2 should indicate a multi-component T2 decay in tissue. Such a multi-component decay has not been observed in experiments carried out in gray matter [28]. However, the ability to detect multi-compartment T2 is dependent upon the absolute difference between compartmental T2 values, exchange between the compartments, and SNR [30]. For example, at the biophysical values used in this work, $P_{\text{mem}} = 0.001$ cm/ms, $T2_{\text{int}} = 25$ ms, and $T2_{\text{ext}} = 150$ ms, the signal decay with TE (sampled at TE = 12.5 by 12.5 to 200 ms) is statistically bi-exponential (F-test rejected at the 0.05 significance threshold) above SNR ~ 200. By increasing P_{mem} to 0.002 cm/ms, the signal decay with TE only becomes bi-exponential above SNR ~ 600. Even with this higher membrane permeability, the trends in Fig. 7 are still observed. ADC decreases with increasing IVF and this decrease is greater at the short $T2_{\text{int}}$ (right panel in Fig. 7) Therefore, it is possible that compartmental differences in T2 could play an important role in filtering compartments in diffusion data, while not exhibiting multi-exponential T2 decay, even at high SNR.

Many models of restricted diffusion have predicted a decrease in the ADC of water with increasing diffusion time [22,25,31], but clinical and experimental results have shown a relatively constant ADC with diffusion time [32]. Figs. 5 and 6 show conditions at low D_{int} and low $T2_{\text{int}}$ for which the ADC is effectively independent of diffusion time. While low D_{int} has been previously hypothesized, allowing a low $T2_{\text{int}}$ further decreases the diffusion time dependence of the ADC, consistent with experimental results.

The strong decrease in ADC with IVF predicted by the present model agrees with previously published models [13,25]. Furthermore, the present model shows that the percentage decrease is strongly dependent upon the difference in T2 between the two compartments. For instance, at $\Delta = 50$ ms, an increase in the IVF from 0.8 to 0.9 reflects only a 24% decrease in the ADC when the T2 is matched between the intracellular and extracellular compartments. However, at the shorter $T2_{\text{int}} = 25$ ms, the same increase in IVF produces a 42% decrease in ADC. This dependence upon $T2_{\text{int}}$ is due to the decreased dependence of the ADC on unrestricted diffusion in the extracellular space as the cell swells to a higher volume fraction. Similarly, Vestergaard-Poulsen *et al.* predicted a large decrease in ADC

with low $T2_{\text{int}}$ compared to $T2_{\text{ext}}$, resulting in a 45% decrease while increasing the cell volume fraction from 0.81 to 0.96.

Application to ischemia

The primary observation of DWMRI after ischemia is a 30-50% decrease in the ADC of tissue water. Several changes in biophysical properties have been hypothesized to explain this observation. Table 1 presents a few possible scenarios of cellular changes in response to ischemia, and the predicted drop in ADC calculated from the FD model. In tissue, the IVF has been measured to increase from approximately 0.8 to between 0.9 and 0.95 during ischemia [18,19]. When cells swell at the expense of the extracellular space, the increase in IVF alone is responsible for an ADC decrease of 32.3% when $T2_{\text{int}} = 50$ ms and 42.4% when $T2_{\text{int}} = 25$ ms. A change in IVF would also tend to change the $T2$ from the global tissue measured by MRI. Because such a change in $T2$ is not observed experimentally, we have raised the $T2_{\text{int}}$ such that the apparent $T2$ is the same before and after swelling. Such an increase could result from the dilution of the intracellular space because of cell swelling. This only accounts for an additional 1.5-3.1% decrease in the ADC. As discussed earlier, inducing changes in D_{int} minimally affects the ADC, causing only an additional $\pm 2\%$ change in the ADC with a decrease in D_{int} from 1.0 to $0.7 \mu\text{m}^2/\text{ms}$ or an increase from 1.0 to $1.2 \mu\text{m}^2/\text{ms}$. The model also predicts that changes in the ADC with membrane permeability depend upon the difference in compartmental $T2$. An increase in membrane permeability, P_{mem} , to 0.02 cm/s could account for an additional 3.3% decrease in ADC when $T2_{\text{int}} = 25$ ms, while at $T2_{\text{int}} = 50$ ms the change in ADC decreases by 5.4%.

The present model predicts that, although many factors may play a role in the decreases in ADC observed following ischemia, a change in the IVF due to cell swelling is a dominant factor. When compartmental differences in $T2$ are present, cell swelling is sufficient to account for the 30-50% decrease in ADC observed after ischemia. Other modeling results have been interpreted to limit the effects of IVF on the ADC. For instance, Ford *et al.* predicted little decrease in the ADC with cell swelling when modeling diffusion perpendicular to infinitely long axonal fiber bundles [33]. However, Ford *et al.* neglected compartmental $T2$ differences, which is required in the present model to reach the 30-50% observed in acute stroke. Also, the ADC perpendicular to axon fiber bundles has been shown to be less sensitive to ischemia than the mean diffusivity in isotropic tissue [34].

The effect of cell swelling on the measured ADC has also been studied experimentally. Van Pul *et al.* reported a 20% drop in ADC due to osmotic swelling (80% of normal osmolarity) of perfused hippocampal slices, and a 40% drop in ADC when similar slices were oxygen and glucose deprived [35]. Similarly, Buckley *et al.* found only a 19% decrease in ADC after treating hippocampal slices with ouabain, which induces cell swelling by shutting down the Na^+/K^+ ATPase without altering metabolic activity [27]. At first glance, these results seem to diminish the impact of cell swelling on measured ADC. However, an important detail of these studies is that they are performed at very short diffusion times, $t_{\text{diff}} = 7$ ms ($\Delta = 8$ ms, $\delta = 3$ ms) and $t_{\text{diff}} = 10$ ms ($\Delta = 11.3$ ms, $\delta = 4$ ms) respectively. At such short diffusion times, the present model predicts a small percentage decrease in ADC with increasing IVF, as shown in Fig. 7. Also, a decrease in D_{int} , due to reductions of energy dependent intracellular motion, is predicted to have a much larger influence on the ADC at short diffusion times, as shown in Fig. 5. Therefore, this model predicts that reductions in the ADC following ischemia, measured at very short diffusion times, would be heavily weighted towards changes in D_{int} and less sensitive to changes in IVF, consistent with these experimental observations.

The intracellular diffusion coefficient is an important parameter, as many groups hypothesize that it plays a role in the decrease in ADC observed following ischemia [5,6].

Many indirect measurements on intracellular metabolites and ions have led to the hypothesis that the intrinsic diffusivity of intracellular water decreases after ischemia, and is the driving force behind the decrease in the ADC of water [5,36]. Given the experimental evidence that IVF and D_{int} are both affected in ischemia, this model agrees with the partial and complete decrease in ADC with cell swelling and ischemia, respectively, as observed by van Pul *et al.* and Buckley *et al.*, as well as the ADC drop after ischemia at short diffusion times measured by Does *et al.* [37]. However, recent work in cell cultures has shown an increase in the ADC of intracellular water after ischemia [38], which may indicate that trends in the ADC of water are not be coupled to the ADC of intracellular metabolites and ions at clinical diffusion times. This agrees with our model which predicts that, at more clinically relevant diffusion times ($\Delta > 30$ ms), reductions in ADC would be insensitive to changes in D_{int} and more sensitive to changes in IVF.

While providing insight into the biological properties affecting water diffusion in tissue, and their influence on measured ADC, the model has limitations. While physiologically appropriate values for cell volume fraction and cell size were used, precise values for D_{int} , D_{ext} , $T2_{\text{int}}$, $T2_{\text{ext}}$, and P_{mem} for cells in gray matter are not known. Because the results of modeling are sensitive to these parameters, particularly $T2_{\text{int}}$ and $T2_{\text{ext}}$, the predictive value of this work may be limited by the accuracy of these parameters. Furthermore, the inherent complexity of gray matter, such as variations in cell shape, size, and density are ignored, and the model neglects the presence of any dendrites, unmyelinated axons, and axon terminals. Further work is needed to explore the effects of these factors on the measured ADC in tissue.

Conclusions

In this work, clinically observed DWMRI results have been predicted using physiologically realistic parameters by a mathematical model that incorporates the effects of membrane permeability as well as differential diffusion coefficients and T2 relaxation times between intracellular and extracellular spaces. The model predicts that the biophysical parameters influencing ADC measurements are highly dependent on the diffusion times at which experiments are carried out. At longer, clinically relevant, diffusion times the ADC is very sensitive to changes in the IVF and insensitive to D_{int} . At short diffusion times, however, the ADC is less sensitive to IVF and more sensitive to D_{int} . Using the model, the decrease in ADC following ischemia measured clinically can be fully accounted for by cell swelling alone when the T2 relaxation time is longer in the extracellular spaces than within the cells.

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Figure 1.

Schematic of the FD model geometry. (a) Tissue is modeled by cubic cells separated by extracellular space. The simulation boundary is extended in the diffusion direction such that an insignificant amount of water reaches the boundary during the simulation, while perpendicular boundaries are reflective due to geometric symmetry. (b) Cells are overlaid on a Cartesian mesh of grid points on which Eqn. 1 is approximated. The model considers seven tissue parameters including cell size (L_{cell}), cell separation (L_{sep}), membrane permeability (P_{mem}), intracellular and extracellular diffusivities (D_{int} and D_{ext}), and intracellular and extracellular T2 relaxation times ($T2_{int}$ and $T2_{ext}$).

Figure 2.

Achematic of the diffusion weighted spin-echo pulse sequence considered in the FD model. Signal decay occurs due to molecular diffusion between the two diffusion gradients, while T2 decay occurs throughout the experiment.

Figure 3.

Total signal and intracellular signal vs. b-value at various membrane permeabilities. Simulation results (symbols) for total and intracellular signal decay correspond to an exponential decay (solid line) in the case of free diffusion, while the intracellular signal decay also satisfies the Tanner *et al.* model (dotted line) in the case of impermeable membranes.

Figure 4.

Calculated ADC of water plotted against membrane permeability. Lines connect simulations with identical diffusion times. Panels depict simulation results with a combination of parameters $D_{\text{int}} = 1.0$ and $3.0 \mu\text{m}^2/\text{ms}$, and $T_{2\text{int}} = 150, 50$, and 25 ms. In each case, $D_{\text{ext}} = 3.0 \mu\text{m}^2/\text{ms}$, and $T_{2\text{ext}} = 150$ ms. Shaded regions highlight the physiologically relevant values of membrane permeability.

Figure 5.

Calculated ADC as a function of intracellular diffusivity, D_{int} , at different diffusion times. Simulations show that the ADC is relatively independent of D_{int} at clinically relevant diffusion times ($\Delta > 40\text{ms}$). The increase in ADC with lower $T_{2\text{int}}$ is a result of T2 filtering.

Figure 6.

Calculated ADC as a function of intracellular T2 relaxation time, $T_{2_{\text{int}}}$. Simulations show an increase in the ADC with decreased $T_{2_{\text{int}}}$ due to T2 filtering. Changes in D_{int} affect the ADC at short diffusion times, but do not affect the ADC at longer diffusion times.

Figure 7. Calculated ADC as a function of intracellular volume fraction, IVF. IVF is increased by swelling cells at the expense of the extracellular space. Simulations show that the ADC decreases with IVF, which is more pronounced at low T2_{int}.

Figure 8.

Calculated ADC as a function of TE. Different panels show results at different diffusion times, while lines are connected between ADCs with identical cell properties. T2 filtering is observed as an increase in ADC with TE. The increase is more pronounced with larger differences between intracellular and extracellular T2.

Table 1

Change in ADC due to proposed biophysical effects of ischemia.

Effect	% Decrease in ADC	
	$T_{2\text{int}} = 50 \text{ ms}$	$T_{2\text{int}} = 25 \text{ ms}$
Cell swelling	32.3%	42.4%
Cell swelling + $\uparrow T_{2\text{int}}$	33.8%	45.5%
Cell swelling + $\uparrow T_{2\text{int}}$ + $\downarrow D_{\text{int}}$ / $\uparrow D_{\text{int}}$	35.9% / 33.0%	46.9% / 44.9%
Cell swelling + $\uparrow T_{2\text{int}}$ + $\uparrow P_{\text{mem}}$	28.4%	48.8%