Gray matter blood-brain barrier water exchange dynamics are reduced in progressive multiple sclerosis

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Abstract

Background and Purpose: To compare transcapillary wall water exchange, a putative marker of cerebral metabolic health, in brain T2 white matter (WM) lesions and normal appearing white and gray matter (NAWM and NAGM, respectively) in individuals with progressive multiple sclerosis (PMS) and healthy controls (HC).

Methods: Dynamic-contrast-enhanced 7T MRI data were obtained from 19 HC and 23 PMS participants. High-resolution pharmacokinetic parametric maps representing tissue microvascular and microstructural properties were created by shutter-speed (SS) paradigm modeling to obtain estimates of blood volume fraction (vb), water molecule capillary efflux rate constant (kpo), and the water capillary wall permeability surface area product (PwS ≡ vb·kpo). Linear regression models were used to investigate differences in (i) kpo and PwS between groups in NAWM and NAGM, and (ii) between WM lesions and NAWM in PMS.

Results: High-resolution parametric maps were produced to visualize tissue classes and resolve individual WM lesions. Normal-appearing gray matter kpo and PwS were significantly decreased in PMS compared to HC (p ≤ .01). Twenty-one T2 WM lesions were analyzed in 10 participants with PMS. kpo was significantly decreased in WM lesions compared to PMS NAWM (p < .0001).

Conclusions: Transcapillary water exchange is reduced in PMS NAGM compared to HC and is further reduced in PMS WM lesions, suggesting pathologically impaired brain metabolism. kpo provides a sensitive measure of cerebral metabolic activity and/or coupling, and can be mapped at higher spatial resolution than conventional imaging techniques assessing metabolic activity.

Keywords
blood-brain barrier, cerebral metabolism, DCE-MRI, neurodegeneration, progressive multiple sclerosis

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INTRODUCTION

The pathophysiological mechanisms underlying neurodegeneration in progressive forms of multiple sclerosis (PMS) are not fully understood, but a growing consensus is that chronic inflammation composed of activated microglia, macrophages, and meningeal lymphoid follicles leads to oxidative stress and mitochondrial injury. Mitochondrial injury, in turn, reduces glia-neuron metabolic support, leading to additional oxidative stress, further demyelination, impaired remyelination capacity, oligodendrocyte apoptosis and axonal degeneration, and ultimately neuronal death. Autopsy results show that 20%-40% of chronic lesions in PMS demonstrate ongoing inflammation and tissue destruction. Together, these findings suggest that metabolic deficits driven by mitochondrial abnormalities may be critically important in understanding pathophysiological mechanisms associated with focal (ie, chronic active lesions) and diffuse brain tissue loss in PMS. Because standard metabolic imaging techniques (eg, PET, MR spectroscopy) suffer from poor spatial resolution, identifying a sensitive, reliable, high spatial resolution, in vivo biomarker of brain metabolism is crucial for studying both focal and diffuse metabolic abnormalities.

Brain blood vessel properties, notably the homeostatic water molecule capillary efflux rate constant, are tightly coupled to local metabolic demands. Moreover, transcapillary water exchange kinetics are amenable to mapping at high spatial resolution with shutter-speed-dynamic-contrast-enhanced MRI (SS-DCE-MRI), a two-compartment exchange model that explicitly incorporates the effects of water exchange on the MR signal. With this approach, transcapillary water flux can be estimated via the trans-endothelial water exchange rate constant (kpo) and/or the water permeability surface area product (PwS). PwS is a measure of the total water flux per unit tissue (S is the total capillary surface area per mass [or volume] of tissue), whereas kpo measures the kinetics of water efflux per capillary, and is thus presented as a putative MRI biomarker of brain metabolism.

Here, we used low-dose gadolinium-based contrast agent (GBCA) SS-DCE-MRI to produce high-resolution parametric maps and investigate transcapillary wall exchange kinetics in white matter (WM) lesions, normal appearing WM (NAWM), and normal appearing gray matter (NAGM) in individuals with progressive MS. We hypothesize that water flux across the capillary endothelium is driven by the activities of a large variety of transmembrane proteins, whose kinetics are rate-limited by (and thus synchronized with) those of Na+,K+-ATPase. These, in turn, are responsive to metabolic activity behind the blood-brain barrier (BBB). We thus expect to find reduced transcapillary water exchange in PMS NAWM, NAGM, and WM lesions compared to healthy controls (HC), potentially reflecting metabolic deficits and tissue regions at risk for neurodegeneration.

METHODS

Participants

The study was approved by the local Institutional Review Board; all participants provided written informed consent. Forty-two participants (23 with clinically diagnosed primary or secondary progressive MS and 19 age- and sex-matched HC [Table 1]) were included in this study. Inclusion criteria were age 18-70 years for all subjects, and, for MS subjects, historical diagnosis of primary progressive MS based on 2010 McDonald criteria or current secondary or primary progressive MS, moderate or greater neurological disability from MS with European Database for MS (EDMUS) Grading Scale score ≥ 3.0, either taking no disease-modifying therapy or taking FDA-approved disease-modifying therapy not shown to benefit PMS. Nine PMS subjects were on disease-modifying therapy at the time of imaging (Avonex [n = 4], Copaxone [n = 3], Rebif [n = 1], and Tecfidera [n = 1]).

| TABLE 1 | Study population demographics |
|---------|-------------------------------|
|         | HC (n = 19) | PMS (n = 23) |
| Mean age (years) | 52.7 ± 11.7 | 57.5 ± 6.7 |
| Age range (years) | 34-70 | 43-65 |
| Sex (F/M) | 11/8 | 12/11 |
| Mean disease duration (years) | – | 22.9 ± 9.6 |
| Mean EDMUS EGS | – | 5.5 ± 1.2 |

Note: All the data represents mean ± standard deviation unless otherwise indicated.

Abbreviations: EDMUS EGS, European Database for MS Grading Scale; F, female; HC, healthy control; M, male; n, number of participants.

MRI acquisition

MRI data were acquired with a Siemens MAGNETOM 7T MRI instrument (Erlangen, Germany) equipped with quadrature transmit and 24-channel phased-array receive RF coils. Anatomical imaging included 3-dimensional sagittal T1-weighted magnetization-prepared rapid gradient-echo (MPRAGE) images (inversion time [TI]: 1050 ms; repetition time/echo time [TR/TE]: 2200/3.1 ms; flip angle [FA]: 7°; 0.7 mm isotropic resolution) and 3-dimensional sagittal T2-weighted fluid-attenuated inversion recovery (FLAIR) images (TI/TR/TE: 2150/8000/398 ms; FA: 120°; 0.8 mm isotropic resolution). SS-DCE-MRI data were acquired with a single axial slice inversion recovery (IR) turboflash sequence (TR/TE/FA: 285 ms/1.15 ms/6°; 128 × 96 image matrix; field of view [256 × 192] mm²; 10 mm slice thickness [40 µl nominal voxel size]; 8 TIs 147-1997 ms, 264 ms spacing) positioned in the centrum semiovale. IR-SS-DCE-MRI image sets (2.3 seconds temporal resolution, 50 image sets, 1 min 55 seconds total acquisition time) were obtained before, during, and after injection of 0.014 mmol/kg Gadoteridol (ProHance; Bracco Diagnostics, Cranbury, NJ).

Image processing and statistical analysis

R1 (1/T1) maps were calculated on a voxel-by-voxel basis for each IR image set in the DCE acquisition by fitting the full Bloch equation...
incorporating all RF pulses and delays to the signal magnitude at each TI. Signal inversion recovery was modeled with a two-parameter single exponential, using a gradient expansion algorithm and Levenberg-Marquardt optimization. Pharmacokinetic parametric maps were created by fitting the SS-DCE-MRI data to the two-compartment Shutter-Speed Paradigm (SSP) model as described elsewhere to obtain estimates of $R_{1\text{exv}}$ (the intrinsic extravascular $\text{H}_2\text{O}$ longitudinal relaxation rate constant [sans exchange] corrected for blood contributions), $v_b$ (blood volume fraction), $k_{po}$ (water molecule capillary efflux rate constant), and $P_{w}S = v_{w}k_{po}$, water capillary wall permeability surface area product. The mathematical formulation for the SSP is given in Equation (1):  

$$R_1(t) = \frac{1}{2} \left\{ \left[ R_{1b}(t) + R_{1\text{exv}} + k_{po} + \frac{k_{po}p_b}{(1-p_b)} \right] - \left[ (R_{1\text{exv}} - R_{1b}(t) - k_{po} + \frac{k_{po}p_b}{(1-p_b)})^2 + \frac{4k_{po}^2p_b}{(1-p_b)} \right]^{1/2} \right\}$$  

where $R_{1\text{exv}} = \frac{R_{1}(0)-R_{1b}(0)p_b}{1-p_b}$, $R_{1b}(t) = R_{1b}(0) + r_1(1-h)[\text{CA}_b](t)$, and $p_b = v_b/f_w$.  

Here, $R_{1}(0)$ and $R_{1b}(0)$ are pre-GBCA $R_1$ values of tissue and blood, and $R_1(t)$ and $R_{1b}(t)$ are the measured time-varying $R_1$ values in tissue and blood, respectively; $r_1$ is the gadoteridol relaxivity (3.3 second$^{-1}$ mM$^{-1}$ at 7T); $h$ is the hematocrit (approximately 0.45); $P_{w}$ is the mole fraction of tissue water in blood; $f_w$ is the tissue volume fraction accessible to mobile aqueous solutes (approximately 0.85 in cortical GM, 0.76 in WM, and 0.82 in chronic WM lesions); $\text{CA}_b$ is the GBCA concentration in blood. The venous output function, $R_{1b}(t)$, was obtained from a single voxel placed in the center of the sagittal sinus for each subject, and was temporally aligned with tissue signal to correct for delay in venous GBCA arrival.  

Tissue segmentation masks were created using a bimodal Gaussian fitting of $R_{1\text{exv}}$ histograms as previously described. Masks were eroded and manually corrected to remove lesions and ensure at least 1 voxel gap between WM and GM masks to reduce partial volume contamination. Suspected lesions identified in PMS $R_{1\text{exv}}$ maps were confirmed by corresponding hyperintensity on T2-w FLAIR and hypointensity on T1-w MPRAGE images. Lesions smaller than 2 voxels on $R_{1\text{exv}}$ maps or adjacent to cortex were excluded to minimize partial volume contamination errors. Additionally, lesions identified on T2-w FLAIR or T1-w MPRAGE that were not well-represented on $R_{1\text{exv}}$ maps (ie, smaller than 2 voxels or isointense with surrounding NAWM) were excluded to minimize partial volume contamination. Linear regression models were used to assess the association of $k_{po}$ and $P_{w}S$ in NAGM and NAWM with disease, adjusting for age and sex. A mixed effects model accounting for multiple lesions per patient was used to compare lesions to NAWM in the PMS subjects; p-values were calculated at the .05 significance level in two-tailed tests.

RESULTS

High-resolution parametric mapping with low-dose GBCA

High-resolution parametric maps representing tissue microvascular and microstructural properties were produced using one-seventh the standard clinical GBCA dose. Figure 1 displays single-voxel time course data for GM and sagittal sinus from one PMS subject in panel (A), and the corresponding SSP fit obtained from Equation (1) is shown in panel (B). Spatial resolution was sufficiently high in individual parametric maps to segment GM and WM and resolve individual WM lesions. Representative parametric maps for one HC and the same PMS subject from Figure 1 are shown in Figure 2. These maps demonstrate (i) clear GM and WM definition and contrast on all pharmacokinetic (PK) maps, and (ii) WM lesion conspicuity on $R_{1\text{exv}}$ and $k_{po}$ maps.

Groupwise differences in NAGM and NAWM

Average tissue SS-DCE-MRI parameters obtained from segmented NAGM and NAWM masks in HC and PMS groups are given in Table 2 and are illustrated in Figure 2 box plots. $R_{1\text{exv}}$ is decreased in both PMS NAWM and NAGM compared to HC. $v_b$ was not significantly different between groups in NAGM or NAWM. In NAGM, $k_{po}$ was significantly reduced in PMS compared to HC ($p = .01$) subjects. In NAWM, $k_{po}$ was reduced by 14% in PMS compared to HC groups, but the difference was not significant ($p = .06$). In contrast to NAWM, $P_{w}S$ was significantly decreased in NAGM in PMS compared to HC ($p = .007$) groups. There was no significant effect of age, sex, or PMS subtype on any parametric estimate. Similarly, no associations were found between parametric estimates and EDMUS score or disease duration in the PMS group.

WM lesion abnormalities

Twenty-one lesions identified on $R_{1\text{exv}}$ parametric maps corresponding to hyperintensities on T2-w FLAIR images were found in 10 MS participants (5 male, 5 female; 1-3 lesions/subject). WM lesions were significantly different from PMS NAWM: $R_{1\text{exv}}$ and $k_{po}$ were significantly decreased ($p < .0001$ for both parameters), and $v_b$ was significantly increased ($p = .036$). However, estimates varied widely across lesions, with both ranges and standard deviations exceeding those in NAGM and NAWM. None of the lesions in this analysis showed GBCA enhancement.

DISCUSSION

Chronic inflammation in MS impairs mitochondrial function and induces oxidative stress resulting in mitochondrial injury and metabolic deficiencies in both oligodendrocytes and neurons, promoting demyelination and neuronal death. Detecting mitochondrial dysfunction
BBB water flux is reduced in MS gray matter

**FIGURE 1**  Single-voxel normal appearing gray matter (NAGM; yellow, inset) and sagittal sinus (red, inset) $R_1 (\equiv 1/T_1)$ data from a 61-year-old with progressive multiple sclerosis (maps shown in Figure 2). Panel A illustrates the temporally aligned tissue (blue circles) and blood (red diamonds) response curves as a function of time during the shutter-speed dynamic-contrast-enhanced MRI acquisition (note left and right $y$-axes for tissue and blood, respectively). The corresponding $R_{1b}$ versus $R_1$ ($R_1$ in tissue) plot and associated Shutter-Speed Paradigm fitted curve (solid black line) obtained from Equation (1) are shown in panel B.

**TABLE 2** Tissue average (± standard deviation) shutter-speed pharmacokinetic parameters

|                | HC        | PMS       | Difference | p-value |
|----------------|-----------|-----------|------------|---------|
| **GM**         |           |           |            |         |
| $R_{1\text{exv}}$ (s$^{-1}$) | 0.613 ± 0.036 | 0.562 ± 0.041 | −8%  | .0001   |
| $v_b$ (ml/g)   | 0.049 ± 0.008 | 0.054 ± 0.012 | +9%  | .17     |
| $k_{po}$ (s$^{-1}$) | 1.98 ± 0.42   | 1.64 ± 0.41  | −18% | .01     |
| $P_{wS}$ (ml/g/s) | 0.095 ± 0.012 | 0.084 ± 0.013 | −11% | .007  |
| **WM**         |           |           |            |         |
| $R_{1\text{exv}}$ (s$^{-1}$) | 0.915 ± 0.023 | 0.857 ± 0.041 | −6%  | <.0001  |
| $v_b$ (ml/g)   | 0.017 ± 0.002 | 0.019 ± 0.003 | +9%  | .06     |
| $k_{po}$ (s$^{-1}$) | 3.28 ± 0.85   | 2.83 ± 0.70  | −14% | .06     |
| $P_{wS}$ (ml/g/s) | 0.055 ± 0.011 | 0.052 ± 0.009 | −6%  | .29     |
| **WM lesions** |           |           |            |         |
| $R_{1\text{exv}}$ (s$^{-1}$) | −        | 0.522 ± 0.062 | −39% | <.0001  |
| $v_b$ (ml/g)   | −        | 0.034 ± 0.023 | +78% | .036    |
| $k_{po}$ (s$^{-1}$) | −        | 1.93 ± 0.71  | −33% | <.0001  |
| $P_{wS}$ (ml/g/s) | −        | 0.063 ± 0.038 | +20% | .2      |

aCompared to normal-appearing white matter in all participants with progressive multiple sclerosis.

Abbreviations: GM, gray matter; HC, healthy control; $k_{po}$, water molecule capillary efflux rate constant; PMS, progressive multiple sclerosis; $P_{wS}$, water capillary wall permeability surface area product ($\equiv v_b \times k_{po}$); $R_{1\text{exv}}$, extravascular $^1$H$_2$O relaxation rate constant; $v_b$, blood volume fraction; WM, white matter.

In vivo is important in understanding and monitoring subtle and potentially reversible disease activity in PMS. In this study, we find reduced transcapillary water exchange in PMS WM lesions, NAWM and NAGM, compared with healthy controls. We also find decreased $R_{1\text{exv}}$ in both PMS NAWM and NAGM compared to HC, reflecting reduced extravascular, intracellular macromolecular content.

Decreased transcapillary water flux could reflect a cascade of events resulting from chronic inflammation, affecting energy metabolism and ion homeostasis. The Na$^+$,K$^+$-ATPase is a ubiquitous membrane protein that is central in the establishment of ion gradients across cell membranes. Intra- and extracellular ion gradients provide the driving force for cellular work, such as substrate transport across cell membranes. After an action potential, Na$^+$,K$^+$-ATPase removes intracellular sodium in an energy-dependent process. In myelinated axons, the nodes of Ranvier are enriched with Na$^+$,K$^+$-ATPase, sodium channels, and mitochondria to facilitate relatively low-energy saltatory conduction of action potentials. However, these ion channels are redistributed after demyelination to allow the continuation of action...
Mitochondria are transported to axons from neuronal cell bodies to meet the increased energy demand. Free radical damage, potentially associated with chronic background inflammation, leads to mitochondrial and nuclear DNA mutations, mitochondrial protein degradation and lipid oxidation, and ultimately mitochondrial dysfunction. When the compensatory axonal mitochondrial response becomes exhausted, energy demand exceeds energy supply and sodium accumulates in the axoplasm due to Na\(^+\),K\(^+\)-ATPase dysfunction. In addition to causing impaired nerve transmission, intracellular sodium accumulation triggers reverse Na\(^+\)/Ca\(^{2+}\) exchange, which can result in calcium overload that in turn activates autolytic proteases and leads to neuronal cell death and apoptosis. In addition to moving ions, membrane ion transporters also move water; it has been estimated that for each ion moved by the Na\(^+\),K\(^+\)-ATPase, 800 water molecules are also transported. This finding indicates that water flux across the capillary endothelium is driven by local tissue homeostatic ion pump activity, including Na\(^+\),K\(^+\)-ATPase, 2Cl\(^-\), and Na\(^+\),K\(^+\)-ATPase metabolic turnover, which in turn is responsive to metabolic activity behind the BBB. The lower the metabolic activity behind the BBB, the slower the turnover of endothelial membrane proteins associated with substrate transport and the slower the accompanying water flux. Thus, it is the tight metabolic coupling within the neurogliovascular unit that allows assessment of metabolic activity with k\(_{po}\) determined by SS-DCE-MRI at the capillary endothelial level.

A variety of multiple sclerosis neuroimaging studies have revealed diffuse abnormalities in normal appearing cortical gray matter and damage can be significant even when lesion accumulation in the white matter is minimal. Consistent with these reports, our finding of reduced k\(_{po}\) in PMS NAGM likely reflects metabolic deficits resulting...
from widespread lipid oxidative injury and energy failure in cortical NAGM. Because NAGM $v_{b}$ is not different between groups, it is likely that $P_{wS}$ differences are driven by $k_{po}$.

Although NAWM $k_{po}$ differences between groups did not reach statistical significance, studies with a larger cohort and whole-brain SS-DCE-MRI may reveal significant differences in NAWM. Decreased $P_{wS}$ has been reported in NAWM in a small cohort of relapsing-remitting MS ($n = 11$) subjects compared to HC ($n = 14$). Slight NAWM PMS $k_{po}$ decreases and $v_p$ increases in PMS combine to produce $P_{wS}$ values that are not different from HC at the group level in this study. This finding may reflect a pathological distinction between relapsing and progressive forms of MS but will need to be investigated further in larger cohorts. In contrast, Figure 2 parametric maps illustrate an individual with PMS in which NAWM $v_b$ does not increase to compensate for decreased $k_{po}$, and thus $P_{wS}$ is also reduced compared to HC in this subject.

Noncontrast MRI techniques based on diffusion-weighted (DWI) and arterial spin labeling (ASL) MRI have been used to map whole-brain $P_{wS}$ and $E_w$ (water extraction fraction, related to $k_{po}$) in relapsing-remitting MS. Consistent with the findings in the present study, decreased $P_{wS}$ was observed in chronic (non-GBCA-enhancing) MS lesions. However, all analyzed lesions were averaged together for each subject, effectively ignoring the possibility of lesion heterogeneity and masking a potentially important application of these techniques. The high degree of PK parameter variability between lesions observed in the present study may reflect lesion pathology heterogeneity (ie, a mixture of smoldering and chronic-inactive lesions), and thus may present a novel biomarker of inflammatory activity in nonenhancing WM lesions. DWI/ASL techniques have the important advantage of not requiring GBCA but are currently limited by inherently poor signal to noise ratio and thus require large ($\sim 64 \mu l$ voxels and long acquisition times (10-20 minutes or more). Only the $k_{po}$ factor (related to $P_{wS}$) measures water "permeation"; the $v_p$ factor (related to $S$) does not. Indeed, local blood volume may change in response to MS pathology or capillary dysfunction, and thus the composite $P_{wS}$ measurement may be blind to important pathological $k_{po}$ changes.

We see the latter effect in our results (Table 2; Figure 2). Reflected as insignificant $P_{wS}$ changes from HC WM to PMS NAWM to lesion, $v_p$ changes (particularly lesion "increases") are obscuring noticeable $k_{po}$ "decreases" (particularly in lesions). Analogous remarks can be made for the GM $P_{wS}$ changes we find from HC to PMS.

We have recently used this experimental approach to report WM $k_{po}$ decreases with HC aging. Other investigators have reported BBB water permeability increases in rat brain Alzheimer’s disease (AD) models. Though the researchers used the same method as here, and measured the $k_{po}$ and $v_p$ factors separately, they report only the composite $P_{wS}$ products. Thus, it is impossible to know if water permeation does actually increase in AD. Because the very large HC water BBB permeability is metabolically controlled, it seems unlikely it would increase in pathology.

It is important to recognize that BBB permeabilities to water and GBCA differ by six orders of magnitude and do not reflect the same underlying processes, or when altered, pathologies. Increased BBB permeability to GBCA primarily reflects increased passive transcellular transport due to loss of tight junction integrity and although rate constants for these transcellular pathways increase by up to three orders of magnitude in active MS lesions compared to NAWM, in absolute terms these rate constants are still quite small compared to values for water. Although water permeability will increase through broken tight junctions, the relative increase is small because water enjoys relative high permeability across cell membranes from both passive and active processes.

The principal limitation of the present study is the limited coverage provided by the single-slice acquisition. Single-slice IR-SS-DCE-MRI was employed for this study to ensure high-fidelity pharmacokinetic modeling at the expense of limited brain coverage and increased sensitivity to motion. Through-plane motion cannot be corrected in single-slice data, and may prevent reliable parametric mapping. Alternatively, region of interest or tissue average fittings, as previously reported, are less sensitive to motion artifacts and are useful in certain applications. Additionally, small lesions or those only partially contained within the centrum semiovale section used here may be included in NAWM masks. Preliminary results demonstrating feasibility of high-spatial resolution (8-20 $\mu l$ voxels) 3-dimensional SS-DCE-MRI acquisitions have been presented and will be used in future studies to overcome the limitations imposed by the single-slice acquisition in the present work.

Transcapillary water exchange may provide a window into cerebral metabolic activity and/or coupling, can be mapped at higher spatial resolution than conventional metabolic imaging techniques, and shows promise in reflecting PMS metabolic derangements. Similarly, markedly decreased $k_{po}$ in T2-w lesions suggests a hypometabolic state common in chronic inactive MS lesions. Longitudinal studies will determine if $k_{po}$ (and/or $P_{wS}$) is sensitive to potentially hypermetabolic inflammation and microglial activation associated with chronic active lesions, and if decreased NAWM or NAGM $k_{po}$ (and/or $P_{wS}$) is predictive of brain atrophy and clinical progression in PMS.

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