Deep Gray Matter Perfusion in Multiple Sclerosis

Dynamic Susceptibility Contrast Perfusion Magnetic Resonance Imaging at 3 T

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Objectives: To assess the presence of perfusion abnormalities in the deep gray matter of patients with relapsing-remitting and primary progressive multiple sclerosis (MS) in comparison with healthy controls and to investigate the impact of perfusion impairment on clinical disability and fatigue.

Design: Survey.

Setting: Research-oriented hospital.

Patients: Twenty-two patients with MS and 11 age- and sex-matched healthy volunteers.

Intervention: Absolute cerebral blood flow, cerebral blood volume, and mean transit time were measured in the thalamus, putamen, and caudate nuclei.

Main Outcome Measures: Decrease of cerebral blood flow in the deep gray matter of patients with MS and correlation between perfusion impairment and the severity of fatigue.

Results: The cerebral blood flow value averaged over the thalamus, putamen, and caudate nuclei was significantly lower in patients with primary progressive MS (P < .001) and in patients with relapsing-remitting MS (P = .01) compared with controls, and there was a trend for patients with primary progressive MS to have lower average cerebral blood flow than patients with relapsing-remitting MS (P = .06). With respect to cerebral blood volume, there was a significant difference between patients with primary progressive MS and controls (P < .001) and between the 2 groups of patients (P = .03) but not between patients with relapsing-remitting MS and controls (P > .30). The fatigue score was significantly correlated with cerebral blood flow (r = 0.4; P < .001) and cerebral blood volume (r = 0.5; P = .004).

Conclusion: The decrease of tissue perfusion in the deep gray matter of patients with MS is associated with the severity of fatigue.

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Cortical and subcortical gray matter (GM) injury is gaining increasing recognition as a prominent pathological substrate of irreversible disability in multiple sclerosis (MS). Several histological and magnetic resonance imaging (MRI) studies have shown that axons and neurons are affected by the disease and that the damage extends beyond the classic lesions into normal-appearing white matter and GM. Despite this evidence, there is still much to learn about the molecular, cellular, and vascular mechanisms leading to GM injury.

Although an ischemic mechanism has long been acknowledged in histological studies of MS, it is only recently that advances in MRI techniques have allowed for investigations of this phenomenon. Inflammation may result in microvascular damage by different mechanisms. Cytotoxic T cells may recognize their antigen on endothelial cells and activate a clotting cascade, which, in turn, leads to thrombosis. Likewise, specific antibodies may recognize their antigen at the vessel wall and induce vascular damage by complement activation. Furthermore, inflammatory edema may impair microcirculation through focal tissue swelling, whereas exudation of inflammatory cells and intravascular fibrin deposition may induce acute and chronic venous obstructions. In addition, obliterator vasculitis might result in chronic ischemia through the modulation of vascular tone and cerebral blood flow. It is possible, therefore, that perfusion abnormalities contribute to neuroaxonal degeneration, which, in turn, leads to disability.

The assessment of cerebral hemodynamics has been made possible through MRI by using dynamic susceptibility contrast-enhanced (DSC) T2*-weighted MRI. This technique uses signal changes that accompany a tracer passing through...
the cerebral vasculature, which enable a quantitative estimation of cerebral blood flow. Dynamic susceptibility contrast-enhanced perfusion MRI has been applied to the study of several neurological diseases, such as stroke, neoplasm, Alzheimer disease, and MS, to demonstrate perfusion impairment in both lesional and structurally intact brain regions.19

Unlike the cortex, deep gray nuclei are technically easier to study with this technique because the quantification of tissue perfusion is less affected by the contamination of blood in adjacent large veins. In addition to the presence of lesions, metabolic and structural abnormalities of deep gray nuclei have recently been shown in studies using quantitative MRI techniques.20–23 Since the thalamus and basal ganglia (BG) are also critical links of cortical-subcortical circuits controlling several neurologic domains, their injury or dysfunction may lead to sensorimotor, cerebellar, and cognitive deficits as well as fatigue. The latter has been reported to occur in 78% to 87% of patients with MS, and it has been related to the involvement of subcortical GM.24,25

We hypothesized that decreased perfusion in deep GM would reflect neuronal/axonal dysfunction and therefore would be related to neurological deficits and fatigue. We also questioned whether tissue perfusion changes would be more severe in patients with primary progressive (PP) MS than in those with relapsing-remitting (RR) MS because of the predominant neurodegeneration in the former. To this end, we used DSC perfusion MRI at 3 T to determine the presence and extent of perfusion abnormalities in the deep GM of patients with MS in comparison with healthy controls. We also investigated the functional correlates of clinical disability and MS-related fatigue.

METHODS

SUBJECTS

Twenty-two patients with MS meeting the McDonald criteria26 were prospectively enrolled in the study. Eleven patients (3 male and 8 female) had an RR course and 11 patients (7 male and 4 female) had a PP course. Disability was assessed by a single experienced neurologist blind to the MRI findings using the Expanded Disability Status Scale (EDSS) score27 within 1 week of MRI. Fatigue was assessed by a psychologist blind to the MRI findings using the Multidimensional Fatigue Inventory (MFI),28 which consists of a 20-item self-report measure assessing 5 dimensions of fatigue: general fatigue, physical fatigue, reduced activity, reduced motivation, and mental fatigue. Each subscale is the sum of 5 items, which are rated on a 5-point scale ranging from 1 (no, not true) to 5 (yes, that is true). Half of the items in the questionnaire are reverse scored to prevent response pattern bias. Subscale scores range from 4 to 20, with lower scores indicating a higher level of fatigue. In addition to MFI, the patients were also assessed with the depression subscale of the Brief Symptom Inventory (BSI).29 This scale assesses clinical indications of depression such as dysphoric mood and affect as well as reduced motivation and a loss of interest. T scores of 63 or greater indicate a clinically significant level of depression that is equal to the 91st percentile or higher. The mean (SD) values of each of the 5 scores of the MFI and the depression subscale of the BSI are compiled for each group of subjects in Table 1.

Patients were not included if they had corticosteroid use or re-lapses within 4 weeks prior to MRI. The patients with PP MS had a mean age of 53.63 years (range, 29-71 years), median disease duration of 4 years (range, 1-19 years), and median EDSS score of 4 (range, 3-7). The patients with RR MS had a mean age of 46.18 years (range, 31-71 years), median disease duration of 5 years (range, 1-13 years), and median EDSS score of 1.0 (range, 0.0-6.5). Only patients with RR MS were undergoing immunomodulatory treatment with interferon beta-1a (Avonex; Biogen, Cambridge, Mass, or Rebif; Serono, Rockland, Mass) and glatiramer acetate (Copaxone; Teva, Petah Tiqvah, Israel). For comparison, 11 age- and sex-matched healthy controls (4 male and 7 female) were recruited. Their mean age was 50.82 years (range, 29-65 years). Approval for this study was obtained from the Institutional Board of Research Associates of New York University Medical Center and informed consent was obtained from all subjects. Demographic and clinical characteristics of the 3 subject groups are given in Table 1.

MRI ACQUISITION

Magnetic resonance imaging was performed using a 3-T scanner (Trio; Siemens Medical Systems, Erlangen, Germany) with an 8-channel phased-array head coil. The following sequences were collected in all subjects during a single MRI session: (1) dual-echo turbo spin echo (repetition time [TR]=5500 milliseconds; echo time [TE]=12/99 milliseconds; 96 contiguous, 3-mm-thick axial slices with a 256×203 matrix and a 220 mm×190 mm field of view [FOV]; in-plane voxel size, 0.83 mm×0.92 mm; parallel imaging acceleration factor of 2); (2) gradient-echo echo-planar imaging (TR=1000 milliseconds; TE=32 milliseconds; 10 contiguous, 3-mm-thick axial slices with a 128×128 matrix; 220 mm×220 mm FOV; flip angle, 30°; signal bandwidth, 1396 Hz/pixel; in-plane voxel size, 1.7 mm×1.7 mm). Dynamic susceptibility contrast-enhanced MRIs were acquired during the first pass of a standard-dose (0.1 mmol/kg) bolus of gadopentetate dimeglumine (Magnevist; Berlex Laboratories, Wayne, NJ). Contrast was injected at a rate of 5 mL/s, followed by a 20-mL bolus of saline also at a rate of 5 mL/s. A total of 60 images were acquired at 1-second intervals, with the injection occurring at the fifth image so that the
bolus would typically arrive at the 15th to 20th image. (3) Post-
gadolinium T1-weighted spin echo (TR=471 milliseconds; TE=12 milliseconds; 50 contiguous, 3-mm-thick axial slices with a 256×205 matrix and a 220 mm×220 mm FOV; in-
plane voxel size, 0.85 mm×1.07 mm).

**IMAGE PROCESSING AND EVALUATION**

Data were transferred to a Linux workstation for offline per-
fusion analysis using programs developed in-house using the
IDL (interactive data language) programming languages. In all
cases, contrast agent concentration, C, is first found using the
simple relationship

\[ C \propto -\ln \left( \frac{S}{S_0} \right) \]

where \( S \) is the signal intensity and \( S_0 \) is the prebolus signal in-
tensity averaged between acquisitions 2 and 11. This equation as-
sumes that T1 shortening effects are negligible, which is true in
practice since we use a relatively low flip angle to minimize satu-
ration.

Absolute cerebral blood volume (CBV), cerebral blood flow
(CBF) (Figure 1), and mean transit time (MTT) were calcu-
lated using the method of Rempp et al.31 These parameters can
be calculated from the following equations:

\[
\text{MTT} = \frac{\int C \, dt}{C_{\text{max}}},
\]

\[
\text{CBV} = \frac{\int C \, dt}{\int \text{AIF} \, dt},
\]

\[
\text{CBF} = \frac{\text{CBV}}{\text{MTT}}
\]

where \( C \) is the tissue concentration following an ideal, instan-
taneous bolus and \( C_{\text{max}} \) is the maximum value of \( C \). The bolus
is not instantaneous, of course, but an approximation to the
idealized response can be found by deconvolving the mea-
sured tissue concentration with the arterial input function (AIF).
Deconvolution of the measured value of \( C \) by the AIF was per-
formed by standard Fourier methods. Before deconvolution,
gamma variate functions were fitted to both measured curves
(\( C \) and AIF) to reduce noise and to correct for any leakage due
to blood-brain barrier disruption (although in this study we ex-
pected little or no contrast extravasation). The AIF was found
using an automated method similar to that described by Rempp
et al31 and Carroll et al.32 The minimum signal, corresponding
to the bolus peak, is found in each pixel within the head. The
average signal drop and average bolus arrival time are then cal-
culated for all pixels. Pixels where the bolus arrives early and
where the signal drop is larger than average are assumed to be
within arteries. The AIF was calculated by averaging the sig-
nals from the 10 pixels in the scanned region that met these 2
criteria and demonstrated the largest signal drop.

Mean transit time, CBV, and CBF were calculated in regions
of interest (ROIs) in the following brain regions: the thalamus,
putamen, and the head of the caudate. To optimize repro-
cducibility, CBV, CBF, and MTT measurements were taken in 2 ROIs
each in the 3 regions. Regions of interest were fixed in size
(radius of 1 image pixel, 1.7 mm) and were placed to avoid ar-
terial and venous structures. Regions of interest were placed in
the same section positions in patients and controls. Within these
section positions, ROIs were placed in the same locations within
the 3 brain regions described earlier. Perfusion measurements
were obtained by 2 of us (M.I. and S.P.) with more than 5 years
of experience with this type of measurement in clinical and re-
search settings. To exclude interobserver and minimize intraob-
server variability as to the location of ROI placement between
patients, each patient data set was reviewed by both of us at
the same time, as described elsewhere.33 Since all MRI sequences
were centered to the same position, regardless of the number of slices,
the ROIs were placed on the axial gradient-echo echo-planar
image using the corresponding transverse T2-weighted image as
reference to avoid the inclusion of lesions.

**LESION VOLUME MEASUREMENTS**

T2-hyperintense and T1-hypointense lesion volume measure-
ments were performed by a single trained technician, blinded
to subject identity, using a semiautomated segmentation tech-
nique based on local thresholding (Jim version 3; Xinapse Sys-
tems, Northants, England, http://www.xinapse.com), as de-
scribed elsewhere.34

**STATISTICAL ANALYSIS**

Mixed-model regression was used to evaluate differences among
subject groups in terms of each perfusion measure and in terms
of each of the 5 scores of the MFI scale while adjusting for the
potential confounding effects of age and sex. A separate analysis was conducted for each perfusion measure within each brain region with, in each case, the values observed in the left and right sides of the given region constituting the dependent variable. The regression model included patient age and sex as covariates and subject group and side of measurement as fixed classification factors. The covariance structure was modeled by assuming observations to be correlated or independent when derived for the same subject or different subjects, respectively, and by allowing the error variance to differ across subject groups. In patients with MS, the mixed-model analysis was also used to examine the association of each perfusion measure with EDSS score, MFI score, T2-weighted and T1-weighted lesion volume, and disease duration. A regression analysis was performed to compare subject groups with respect to the average of each perfusion measure over the 3 regions. Within the mixed-model format, the Tukey Honestly Significant Difference Procedure was used to make all pairwise comparisons among subject groups with respect to each perfusion measure while adjusting for age and sex and maintaining the familywise type I error rate for the set of comparisons at or lower than the 5% level. Results associated with a P value between .10 and .05 are reported as statistical trends since they may correspond to real differences or effects that the study was not adequately powered to detect and therefore are worthy of further investigation. All statistical computations were carried out using SAS for Windows version 9.0 (SAS Institute, Cary, NC).

RESULTS

None of the healthy controls showed lesions on T2-weighted and T1-weighted scans. Median T2 lesion volume was 2.83 mL (range, 0.12-27.9 mL) in patients with RR MS and 3.89 mL (range, 0.39-32.91 mL) in patients with PP MS. Median T1 lesion volume was 0.4 mL (range, 0.04-3.20 mL) in patients with RR MS and 0.36 mL (range, 0.04-21.5 mL) in patients with PP MS. Four patients with RR MS and 4 patients with PP MS had deep GM lesions noted on T2-weighted imaging (all patients with RR MS and 2 patients with PP MS had 1 lesion, whereas 1 patient with PP MS had 3 and 1 had 2 lesions). Although 3 patients with RR MS and 3 patients with PP MS had a BSI score of 63 or higher, the difference between patients and controls was not significant (P = .06). However, since there was a trend toward patients having a higher mean level of depression and since depression is known to influence fatigue, BSI score was accounted for as a covariate in analyses to compare patients and controls in terms of fatigue. After adjustment for age and sex and BSI score, scores for MFI general fatigue (P = .02), physical fatigue (P = .03), reduced activity (P = .009), reduced motivation (P = .01), and mental fatigue (P = .01) were significantly lower in patients with MS compared with healthy controls. The 2 groups of patients were different only in terms of MFI reduced motivation score, which was significantly lower in patients with PP MS than in those with RR MS (P = .05).

COMPARISON OF PERFUSION METRICS BETWEEN PATIENTS WITH PP MS AND RR MS AND CONTROLS

After adjustment for age and sex, significant differences were found between the patients with PP MS and the controls with respect to the CBF (P≤.004) and CBV (P≤.009) in each of the 3 regions. Although CBF values were lower in patients with RR MS compared with controls in all 3 regions, significant differences were only found between the patients with RR MS and controls with respect to CBF (P = .03) in the head of the caudate. However, the CBV values of patients with RR MS and controls were not significantly different (P>.20) in any of the 3 regions. After adjustment for age, sex, and disease duration, significant differences were found between the patients with PP MS and RR MS with respect to CBV in the thalamus (P = .01) but not in the putamen and caudate nuclei (P > .20). With respect to CBV, there were significant differences in the thalamus and caudate (P = .03 and .04, respectively) but not in the putamen (P = .09). When CBF, CBV, and MTT values were averaged over regions and sides to generate 1 measure per subject, the average CBF was significantly lower in patients with PP MS (P<.001) and in patients with RR MS (P = .01) compared with controls, and there was a trend for the patients with PP MS to have lower average CBF than the patients with RR MS (P = .06). With respect to average CBV, there was a significant difference between patients with PP MS and controls (P<.001) and between the 2 groups of patients (P = .03) but not between patients with RR MS and controls (P>.30) (Figure 2). There were no significant differences among the 3 groups of subjects in terms of MTT (P>.30 for all comparisons). Results pertaining to group comparisons with respect to average CBF, CBV, and MTT are given in Table 2.

CORRELATIONS BETWEEN PERFUSION METRICS, LESION VOLUMES, DISEASE DURATION, EDSS SCORE, AND MFI SCORE

Neither T2 and T1 lesion volumes nor disease duration correlated with the perfusion metrics from the 3 brain regions. A statistical trend was found when investigating the association between the EDSS score and deep GM CBF value (r = −.27; P = .09) and CBV value (r = −.28; P = .09). After adjustment for BSI score, the MFI reduced motivation score was significantly correlated with CBF (r = 0.4; P<.001) and CBV (r = 0.5; P = .004) (Figure 3). In addition, the MFI reduced activity score was significantly correlated with CBF (r = 0.4; P = .004). There was no significant correlation between MTT and disease duration, EDSS score, MFI score, or either measure of lesion volume.

COMMENT

Dynamic susceptibility contrast-enhanced perfusion MRI performed at a field strength of 3 T provides several advantages relative to that performed at 1.5 T. At 3 T, the intrinsic signal-to-noise ratio is higher and sensitivity to T2* changes due to contrast agent is increased. This can be traded off for either lower-contrast dose or a shorter TE and, hence, a larger number of slices and improved coverage within a fixed TR.

Our observation of reduced CBF and CBV in the deep GM of patients with MS is consistent with several histological and MRI studies suggesting that macroscopic and microscopic structural damage as well as metabolic dysfunction occur in the thalami and BG of patients with
Several direct and indirect mechanisms might have contributed to the deep GM hemodynamic impairment. First of all, MS lesions are frequently found in the thalamus and BG at postmortem examination. However, lesions are often missed on T2-weighted images because of their small size and poor contrast with the surrounding GM. In addition, diminished blood supply has been described in MS plaques. Although macroscopic deep GM lesions were detected in a few of our patients, theoretically, small deep GM lesions, not detected on MRI, could have contributed to the decrease of perfusion. Second, axonal transection in active white matter lesions could lead indirectly to anterograde and retrograde degeneration of axons running within the thalamus. Finally, neuronal loss itself secondary to hypoperfusion, iron deposition, demyelination, or Wallerian degeneration might contribute to CBF decrease via reduction in local metabolic activity.

Patients with PP MS exhibited a more severe CBF and CBV decrease, consistent with the prominent axonal loss described in this subgroup of patients. This suggests that perfusion metrics are a potential marker of neuroaxonal damage. Admittedly, unlike the patients with PP MS, the patients with RR MS were all undergoing treatment with disease-modifying drugs that may have partially contributed to the lesser degree of hemodynamic impairment. Nonetheless, our findings are consistent with those of a previous study reporting hypoperfusion of cortical and subcortical GM especially in patients with PP and sec-

**Table 2.** CBF, CBV, and MTT Values Averaged Over the Deep Gray Matter Nuclei of Patients With PP MS, Patients With RR MS, and Healthy Controls

<table>
<thead>
<tr>
<th></th>
<th>Healthy Controls</th>
<th>Patients With RR MS</th>
<th>Patients With PP MS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CBF, mL/100 g per minute</strong></td>
<td>68.0 (7.0)</td>
<td>62.2 (6.5)</td>
<td>56.0 (8.7)</td>
</tr>
<tr>
<td><strong>CBV, mL/100 g</strong></td>
<td>4.8 (0.6)</td>
<td>4.5 (0.6)</td>
<td>3.6 (1.0)</td>
</tr>
<tr>
<td><strong>MTT, s</strong></td>
<td>4.3 (0.5)</td>
<td>4.3 (0.4)</td>
<td>3.8 (0.9)</td>
</tr>
</tbody>
</table>

Abbreviations: CBF, cerebral blood flow; CBV, cerebral blood volume; MTT, mean transit time; PP MS, primary progressive MS; RR MS, relapsing-remitting MS.

*Values are given as mean (SD) and are adjusted for age, sex, and disease duration.

**Figure 2.** Box plots display the 25% to 75% values (boxes) ± 95% values (whiskers), median values (horizontal lines within boxes), and outliers (*) of mean absolute cerebral blood flow (CBF) value (A) and cerebral blood volume (CBV) value (B) distribution in deep gray matter among healthy controls (CTRL) (empty box), patients with relapsing-remitting multiple sclerosis (RR MS) (gray box), and patients with primary progressive (PP) MS (black box). Only significant P values are reported. Note that the median CBF and CBV values in patients with PP MS are lower than those in patients with RR MS, which, in turn, are lower than those in controls.

**Figure 3.** Mean deep gray matter cerebral blood flow (CBF) value (A) and mean cerebral blood volume (CBV) value (B) as a function of Multidimensional Fatigue Inventory (MFI) reduced motivation score for patients with multiple sclerosis. Lower scores of reduced motivation indicate a higher level of fatigue.
In conclusion, although this study has to be regarded as preliminary since it is based on a relatively small sample of patients, it provides additional evidence for the involvement of deep GM in the pathogenesis of fatigue in MS. Also, it suggests that DSC perfusion MRI may provide functional and metabolic information not accessible with conventional MRI.

REFERENCES


Announcement

Calendar of Events: A New Web Feature

On the new Calendar of Events site, available at http://pubs.american-assn.org/cgi/calendarcontent and linked off the home page of the Archives of Neurology, individuals can now submit meetings to be listed. Just go to http://pubs.american-assn.org/cgi/cal-submit/ (also linked off the Calendar of Events home page). The meetings are reviewed internally for suitability prior to posting. This feature also includes a search function that allows searching by journal as well as by date and/or location. Meetings that have already taken place are removed automatically.