Long-Term Blood–Brain Barrier Permeability Changes in Binswanger Disease

Branko N. Huisa, MD; Arvind Caprihan, PhD; Jeffrey Thompson, BS; Jillian Prestopnik, PhD; Clifford R. Qualls, PhD; Gary A. Rosenberg, MD

Background and Purpose—The blood–brain barrier (BBB) is disrupted in small vessel disease patients with lacunes and white matter hyperintensities (WMHs). The relationship of WMHs and regional BBB permeability changes has not been studied. We hypothesized that BBB disruption occurs in normal appearing WM and regions near the WMHs. To test the hypothesis, we repeated BBB permeability measurements in patients with extensive WMHs related to Binswanger disease.

Methods—We selected a subset of 22 Binswanger disease subjects from a well-characterized larger prospective vascular cognitive impairment cohort. We used 16 age-matched controls for comparison. The abnormal WM permeability (WMP) was measured twice for several years using dynamic contrast–enhanced magnetic resonance imaging. WMP maps were constructed from voxels above a predetermined threshold. Scans from first and second visits were coregistered. WM was divided into 3 regions: normal appearing WM, WMH ring, and WMH core. The ring was defined as 2 mm on each side of the WMH border. WMP was calculated in each of the 3 specific regions. We used paired \( t \)-test, ANOVA, and Fisher exact test to compare individual changes.

Results—WMP was significantly higher in subjects than in controls \((P<0.001)\). There was no correlation between WMH load and WMP. High permeability regions had minimal overlap between first and second scans. Nine percent of WMP was within the WMHs, 49% within the normal appearing WM, and 52% within the WMH ring \((P<0.001; \text{ANOVA})\).

Conclusions—Increased BBB permeability in normal appearing WM and close to the WMH borders supports a relationship between BBB disruption and the development of WMHs. (Stroke. 2015;46:2413-2418. DOI: 10.1161/STROKEAHA.115.009589.)

Key Words: blood-brain barrier ■ leukoaraiosis ■ magnetic resonance imaging ■ permeability ■ white matter

Cerebral small vessel disease (SVD) is the main cause of vascular cognitive impairment and a major cause of ischemic stroke. Increased permeability of the blood–brain barrier (BBB) has been observed in mixed groups of SVD patients. BBB disruption might play an important causal role in SVD, possibly through toxic effects of leaked fluid and blood-derived proteins within the white matter (WM). Plasma proteins, such as IgG, complement and fibrinogen, have been identified in the WM of patients with SVD. Also, there is an increase of the cerebrospinal fluid/serum albumin ratio in patients with vascular dementia, which correlates with the degree of WM hyperintensities (WMHs), suggesting that the abnormal WM might be the site of BBB leakage. More recently, magnetic resonance imaging (MRI) studies in patients, using dynamic contrast-enhanced MRI (DCEMRI), have generated permeability maps showing regions of increased WM permeability (WMP), and an association of WM BBB permeability with the load of WMHs. However, long-term BBB changes in WM and the association of WMHs with regions of altered BBB have not been studied longitudinally.

Binswanger disease (BD) is a progressive form of SVD in patients with chronic hypertension and other associated vascular risk factors that have MRIs characterized by large WMHs on fluid attenuated inversion recovery (FLAIR) and T2-weighted imaging sequences, cognitive impairment, mainly in executive function, gait ataxia, and focal neurological signs. These patients usually progress to dementia of the vascular type. We hypothesized that in patients with BD, the WMP relates to the WMHs load and that the BBB permeability increases as the disease progresses, preceding the growth of new WMHs. We postulate that BBB disruption occurs in different regions over time with increased permeability in normal appearing WM (NAWM) in regions near WMHs. To test these hypotheses, we used DCEMRI to generate longitudinal...
permeability maps to measure long-term changes of the WMP. We further compared regions of abnormally high permeability from the 2 scans and analyzed the distribution of these regions within the WMHs, NAWM, and newly formed WMHs.

Methods

Subjects

From a prospective longitudinal vascular cognitive impairment cohort of 95 subjects, we retrospectively selected a subset of 22 SVD subjects thought to haveBinswanger-like features. Vascular cognitive impairment subjects had a clinical evaluation by a board certified neurologist, neuropsychological studies, brain MRI studies, and cerebrospinal fluid analysis for demyelinating profile, cerebrospinal fluid/albumin ratio, and matrix metalloproteinases. The inclusion criteria for the SVDBinswanger-like subjects were as follows: two completed brain MRI with contrast >10 months apart, large WMHs (Fazekas >2), >2 vascular risk factors, cognitive complaints, and focal neurological symptoms or gait disturbances. We excluded patients with cortical strokes, SVD because of genetic/toxic/metabolic causes and patients with suboptimal DCEMRI (motion artifact). We selected 16 asymptomatic age-matched controls for comparison. In agreement with recently published literature, we retrospectively applied theBinswanger score to our patient selection. All patient selection was blinded to the permeability data. The University of New Mexico Human Research Review committee approved all aspects of the study.

Neuropsychological Testing

Standardized measures of cognitive function were given to all patients in the study. All tests were administered and scored according to standard procedures for that test and were administered by a trained psychologist. Standardized (T) scores were calculated for each test using published norms for each test. Averaged composite T scores were calculated for each of the 3 cognitive areas of interest (memory, executive functioning, and processing speed), as well as an overall composite of cognitive functioning. Tests for each composite included memory (Hopkins Verbal Learning Test-Delay, Rey Complex Figure Test-Long Delay), executive (Digit Span Backwards, Trail Making Test B, Wisconsin Card Sorting-Total errors, or Stroop Color and Word Test-interference score), processing speed (2 subtests from the Wechsler Adult Intelligence Scale–Digit Symbol and Symbol Search). The overall composite included these 3 composites, plus attention (Digit Span Forward and Trial Making Test A) and language (Boston Naming 60 item test, Controlled Oral Word Association [FAS]), averaged.

MRI Acquisition

The study started on the Siemens 1.5T Sonata MRI scanner but is currently being done on the Siemens 3T Trio scanner. Fourteen BD patients were scanned twice on 1.5T MRI and 8 patients on 3.0T; all repeat studies were done on the same instrument used in the first scan. The measurements consist of a structural T1 scan, a FLAIR scan for characterizing WMHs, and a T1 mapping with partial inversion recovery (TAPIR) scan for BBB permeability calculations. TAPIR uses sequential, rapidly acquired, axial T1 measurements with the first image taken before the gadolinium diethylenetriaminepentaacetic acid (Magnevist, Bayer Schering Pharma) injection and multiple T1 measurements after injection. A quarter of the standard dose of gadolinium diethylenetriaminepentaacetic acid is injected with an automatic power injector (Medrad Spectris SolarisVR MR Injection System; Siemens). The scan parameters were similar at the 2 scanner field strengths with slightly higher resolution images at 3T and TAPIR images also having better temporal resolution. The scan parameters are summarized in the Table in the online-only Data Supplement.

Structural Image Analysis

The FLAIR images were segmented for WMHs, using a semiautomated software package (JIM V.6.0, Xinapse Systems Ltd, Northants, UK; http://www.xinapse.com). A single experienced physician blinded to the clinical information, used the semiquantitative Fazekas scale for rating WMHs. The same rater used T1, T2, and FLAIR for grading perivascular spaces and counting lacunar and cortical strokes for each MRI scan.

Permeability Analysis

The voxel-by-voxel permeability maps were calculated from TAPIR measurements based on the Patlak model of Gd-contrast agent leaking through the BBB as described earlier. The T1 images, the FLAIR image, and the permeability maps from each visit were spatially registered to the FLAIR image of the first visit for evaluating longitudinal changes. Permeability was calculated only within WMH and the NAWM regions. The WM was defined by segmenting the T1 image by fast/Functional MRI of the Brain Software Library (FSL; www.fmrib.ox.ac.uk/fsl/), followed by eroding the WM segment by 7 mm to avoid proximity with gray matter and the cerebrospinal fluid. This gave a more consistent permeability calculation in the WM. The regions of active BBB leakage were defined by exceeding a threshold permeability of 0.003/min. The permeability threshold was varied from 0.001 to 0.005/min, and 0.003/min gave the maximum accuracy of correctly predicting controls and patients. The threshold was obtained from the data itself, because of the small sample size. WMP is the sum of all permeability voxels over the active area of BBB leakage. WMP depends on the value of the cutoff threshold.

To understand the relationship of WMP and the development of new WMHs, we calculated the voxel overlap between the initial visit WMP map and the newly formed WMHs from visit 2 (WMH visit 2–WMH visit 1). The overlap was reported as percentage of newly formed WMH voxels with prior abnormal permeability.

We combined data from the first and second visits to study the spatial distribution of the WMP in relation to the WMHs. This was by computing the overlap between the 2 visits for each MRI study, using all 3 fluid-structures: cerebrospinal fluid; CSF, cerebrospinal fluid; EXE, executive; MEM, memory; MRI, magnetic resonance imaging; SPEED, processing speed; and WMHs, white matter hyperintensities.

Table. Comparison of Clinical, MRI, Cognitive, and CSF Factors Between BD Subjects and Controls

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<th>Controls=12</th>
<th>PValue</th>
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<tr>
<td>Age, y</td>
<td>67±10</td>
<td>61±9.5</td>
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<tr>
<td>Sex (female)</td>
<td>41%</td>
<td>44%</td>
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<td>Hypertension</td>
<td>86%</td>
<td>19%</td>
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<tr>
<td>Diabetes mellitus</td>
<td>27%</td>
<td>0%</td>
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<tr>
<td>Focal neurological signs</td>
<td>68%</td>
<td>13%</td>
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<tr>
<td>Previous stroke</td>
<td>50%</td>
<td>0%</td>
<td>0.001</td>
</tr>
<tr>
<td>Ataxia/imbalance</td>
<td>73%</td>
<td>6%</td>
<td>0.001</td>
</tr>
<tr>
<td>MRI features</td>
<td></td>
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<tr>
<td>Lacunes</td>
<td>82%</td>
<td>0%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WMHs*</td>
<td>39472±2474</td>
<td>702±449</td>
<td>&lt;0.001</td>
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<td>1.46±0.77</td>
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</tr>
<tr>
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<td>3.28±1.13</td>
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<td>CSF/albunin ratio†</td>
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<td>5.6±2.8</td>
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<tr>
<td>Cognitive evaluation</td>
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<td>MEM</td>
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<td>SPEED</td>
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<tr>
<td>BS‡</td>
<td>5.8±1.7</td>
<td>1.2±1.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

BD indicates Binswanger disease; BS, Binswanger Score; CSF, cerebrospinal fluid; EXE, executive; MEM, memory; MRI, magnetic resonance imaging; SPEED, processing speed; and WMHs, white matter hyperintensities.

*Supratentorial WMHs volume in voxels.
†CSF from different age-matched controls receiving spinal surgery.
‡BS of >5 have a 78% likelihood of BD.

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done because visual examination of the permeability maps indicated that regions of high permeability clustered at the boundary of the WMHs. In this analysis, we divided the WM into 3 regions of interest: (1) NAWM, (2) 4-mm ring bordering the WMHs constructed by dilating and eroding the WMH mask by 2 mm on each side of its border, and (3) the WMHs within the ring. We calculated the WMP for each of these regions.

Statistics

For group comparison of normally distributed data, we used Satterthwaite Student t test and paired t test for long-term changes of individual variables. For group comparison binomial data, we applied Fisher test and ANOVA for repeated covariate analysis. Statistical analyses were conducted with SAS v12.1 and SPSS v20.0.

Results

Twenty-two subjects with clinical features of BD and 16 age-matched who meet inclusion and exclusion criteria completed brain scans and neuropsychological evaluation at both visits. The Table shows the baseline characteristics of patients and controls. Increased total WMP was seen in all BD subjects in both visits when compared with controls (ANOVA; P<0.001), but no difference between patient visits. Box plots are shown with outliers as circles. WMP variances were higher in BD subjects than in controls (ANOVA, P<0.01).

Control subjects showed little permeability and when it was present, the pattern showed the permeability was scattered randomly around the WM. Figure 3 shows representative WMP maps in the age-matched controls. Occasionally, a small patch of periventricular WMH was seen in the controls.

The overlap of newly formed WMHs with the WMP map from visit 1 showed 11% of new WMHs voxels had prior abnormal permeability. Similarly, WMHs showed 14% of WMHs voxels with abnormal permeability. WMP map in visit 1 was compared with visit 2. Most of the regions showing permeability at visit 1 were gone in visit 2, and only 5±2% of the voxels with increased WMP overlapped between the 2 visits. The regions with increased permeability in visit 1 are shown in 1 color and those in the second visit in another color with the overlapped areas in a third color (Figure 4). After visual inspection of the WMP regions in scans from visits 1 and 2, we observed that the increased permeability seemed to cluster around the edges of the WMHs. To explain the location of the permeability in relation to the WMHs, we drew a series of new regions of interest that consisted of a 4-mm ring, which was traced along all WMH borders. An illustrative BD patient is shown with a rim around the WMHs and the permeability superimposed on the FLAIR image (Figure 5A). The 4-mm rim is drawn in the enlargement of one of the WMHs with the permeability shown in red (Figure 5B). Plots of individual subjects are shown to indicate the rationale for the selection of the rim size. The exponential slope shows the grouping of high permeability voxels within the 4-mm ring. We observed a clustering of the high permeability voxels close to the border of WMHs (Figure 5C). Using the
permeability maps from the 3.0 Tesla patients only, we found that 51% of total permeability voxels were located inside the 4-mm ring, 9% were located within the core of the WMHs, and 49% were located within the NAWM (ANOVA; \( P < 0.001 \)).

**Discussion**

We found that BD patients have increased BBB permeability, which remains elevated after 1 to 2 years. Sites of abnormal BBB permeability on the first scan tended to resolve with new areas arising in the second scan. The volume of WMHs failed to correlate with the WMP. Comparing voxel permeability maps between the 2 scans showed little overlap, suggesting a continuous, but fluctuating, pattern of BBB disruption. The small variability in WMP from controls compared with the high variability in BD patients argues against these changes being spurious and suggests that WMP fluctuations arise from biological processes.

A unique aspect of this study is the ability to follow the regions of high permeability with voxel mapping. The initial WMP scan did not predict future WMH formation mostly because of the small overlap between initial permeability and the new WMHs. We also observed that regions of permeability in the 2 scan rarely overlapped. Original high permeability regions resolved over time with new regions of leakage appearing in other locations. Such variability in permeability might account for the modest overlap found between the first WMP map and second scan.

Because these observed spatial fluctuations in permeability could be attributed in part to coregistration errors, poor spatial resolution, or reproducibility of the imaging technique, we further studied the spatial relationship between WMHs and the BBB permeability maps from each individual scan. By tracing a ring around the WMHs borders, we demonstrated that most high permeability voxels were located either in NAWM or the surrounding edges of the WMHs with only a few voxels seen inside the core of the WMHs. Our results strongly suggest that the WMHs borders obtained on FLAIR do not dictate absolute permeability changes in the WM. Instead, it seems that there is a gradual transition of high WMP from outside of the WMHs core to NAWM regions. These findings are of special interest for understanding the disease progression. Studied using fractional anisotropy (FA), an MRI method that measures the WM microstructure, has shown that a penumbral region of reduced FA surrounds WMHs. Long-term follow-up showed that baseline abnormalities in FA predicted future WMHs formation. These FA changes are larger within regions of enlarging WMHs than in other NAWM regions. Our findings that WM BBB disruption seems to cluster around WMHs might represent another indicator of disease activity associated with local WMHs growth. Defective WM BBB might be an earlier

**Figure 3.** Permeability maps in 4 representative control patients. The normal controls had some areas of white matter hyperintensities (WMHs) mainly in the periventricular regions (arrows). Green delineates the WMHs, The red areas of scattered increased permeability were lower than found in the Binswanger disease patients. None of the controls had regions of high permeability (yellow). The color code used showed increasing permeability from red to yellow.

**Figure 4.** Permeability maps from visit 1 and 2. Examples of 5 Binswanger disease patients (rows) measured twice for a 1-year period showing the different permeability areas from scan in visit 1 to scan in visit 2. White matter hyperintensities (WMHs) are shown in green, high permeability areas in red (visit 1) and blue (visit 2). A, Fluid attenuated inversion recovery; (B) WMHs and permeability at visit 1; (C) WMHs and permeability at visit 2; (D) Combined high permeability maps from visits 1 (red) and 2 (blue). Average overlapped areas represented only 5% on the permeability maps (in yellow).
phenomenon that indicates endothelial dysfunction followed by WM tract abnormalities. This initial BBB disruption in the WM has been observed in the hypertensive stroke prone rat animal model before the appearance of subcortical ischemic changes.\(^{17}\)

Lacunar infarct is another form of SVD responsible for 20% of all ischemic strokes and it is strongly associated with the presence of WMHs.\(^{18}\) There is mounting evidence indicating that the NAWM surrounding the visible WMHs on FLAIR corresponds to regions of future WMHs growth and new lacunar stroke occurrence. This has been observed also in patients with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy, a genetic form of SVD.\(^{19}\) New small diffusion-weighted image lesions occurring at the borders of the WMHs have also been postulated to be related to WMHs growth.\(^{20}\) BBB disruption has been observed in lacunar stroke patients but it is unknown whether it precedes the occurrence of new lacunar infarcts.\(^{2}\) Patients with BD typically have lacunar infarcts mainly discovered incidentally after brain imaging. However, lacunar strokes can also occur in the absence of WMHs, perhaps because of other pathophysiological mechanisms such as intracranial atherosclerotic plaque or microemboli.\(^{21}\) In these instances, the earlier BBB disruption might not play an important role. Therefore, it is possible that lacunar strokes have different pathophysiological mechanisms. Future studies using DCEMRI and permeability maps could differentiate the pathophysiological mechanisms of lacunar strokes.

Our patients have larger WMHs and it might be that these lesions, which represent gliosis, no longer have inflammation related to changes in permeability. Because both DCEMRI and FA demonstrate abnormalities in the NAWM in patients with large WMHs, future use of the combination of these 2 methods needs to be assessed as possible imaging biomarkers for interventional trials.

We also observed an increase of WMP in NAWM distant from the WMHs. Using a different BBB MRI technique, another group has shown in a mixed SVD group of patients an increase of BBB permeability in the NAWM that was associated with the WMH load.\(^{22}\) Our results are consistent with these observations, but we were unable to show an association between the load of WMHs and WMP. Our finding supports the notion that WMP is a migrating process, whereas WMH load is an accumulating process.

Based on the alterations of BBB in the NAWM, and the greater density of high permeability voxels in the rim surrounding the WMHs, we postulate that areas of NAWM with increased permeability are regions vulnerable to growth of WMHs. Furthermore, we found pathological BBB changes occurring in the WM of BD patients in both the NAWM and around the edges of the WMHs, suggesting a diffuse pathological process. Strengths of our study include the first demonstration of regional visualization of the WMP maps. There are several caveats of our study, including small sample size, retrospective design, different scanner strength, suboptimal spatial resolution, and lack of autopsy diagnostic verification.

In summary, our study reveals that patients with BD have a persistent disruption of the BBB that fluctuates between WM regions over time. The increased number of high permeability voxels found in regions around WMHs strengthens the association of BBB disruption with the development of WMHs, but further studies will be needed for confirmation.

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**Disclosures**
None.

**References**


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## Supplemental Table: MRI Acquisition Parameters.

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<td>256</td>
<td>192</td>
<td>1 mm</td>
<td>6000</td>
<td>408</td>
<td>2000</td>
<td>Sagital</td>
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脳小血管病（SVD）は血管性認知機能障害の主原因であり、虚血性脳卒中の大きな要因の1つである。血液脳関門（BBB）の透過性亢進は、さまざまなグループのSVD患者で認められてきた。BBBの破壊はSVDの発症に重要な役割を果たしているとみられ、これは白質（WM）内に漏出した体液および血液由来の蛋白が引き起こす毒性作用によるものと考えられている。SVDの患者の白質ではIgG、補体、フィブリンオーゲなどの血漿蛋白が検出される。また、血管性認知症の患者では脳脊髄液／血清アルブミン比が上昇するが、これは白質高信号変化（WMH）の程度と相関することから、異常な白質部分がBBBの破壊漏出部位と考えられた。近年患者で実施された磁気共鳴画像法（MRI）研究では、ダイナミック造影MRI（DCE-MRI）を使用して、白質透過性（WMP）が亢進した領域だけでなく白質BBBの透過性とWMH容量の関連が分かる透過性マップを作成された。しかし白質におけるBBBの長期変化や、WMHと変化したBBB領域の関連を検討した研究は行われていない。

Binswanger病（BD）は進行性のSVDで、慢性高血圧とそれに関連する脳血管障害の危険因子を持つ患者に発症する。MRI検査ではfluid attenuated inversion recovery（FLAIR）画像およびT2強調画像に巨大なWMHを認め、主に実行機能が低下する認知機能障害、失調性歩行、局所神経学的障害を示す。これらの患者は通常、血管性認知症に移行する。本研究は仮説として、BD患者ではWMPとWMHの容量に関連があり、新しいWMHが発生する前に、疾患の進行に伴いBBBの透過性が亢進すると考えた。BDの破壊の、WMHに近い正常に見える白質（NAWM）において異なる時期に異常な領域で透過性の亢進に伴って起こると推測される。上記の仮説を検証するため、DCE-MRIでWMPの長期変化を測定する繰延的透過性マップを作成した。さらに、2つの画像で透過性が異常亢進した領域を比較し、WMH、NAWM、新しく形成されたWMH内でこれら領域の分布を解析した。

方法

症例

血管性認知機能障害の前向き継続コホート95例から
ら、Binswanger様の特徴を示すと考えられたSVD患者22例を後向きに選択した。血管性認知機能障害の被験者には、専門医資格を持つ神経内科学科による臨床評価、神経心理学的検査、脳MRI検査、脳脊髄液検査による脳橋疾患の特性、脳脊髄液／アルブミン比、マトリックスメタプロテインの測定を実施した。SVD Binswanger様被験者の選択基準は、11ヶ月以上の間隔をあけて脳造影MRI検査を2回実施した、大きなWMHがある（Fazekas＞2）、脳血管障害の危険因子を3個以上有する、認知機能の訴えがある、局所神経学的または歩行障害がある症例とした。皮質梗塞、遺伝／毒物／代謝に起因するSVD患者およびDCIMRI画像が適切でない（体動によるアーチファクトが溶ける）患者は除外した。比較のため、年齢が一致する無症状の対照16例を選択した。近年発表された文献に従って、本研究の症例選択にBinswangerスコアを後向きに使用した。症例選択は遺伝性データが有ければ行った。ニューメンシコン大学のヒューマンリサーチ審査委員会が研究を全面的に承認した。

神経心理学的検査

本研究の患者すべてに標準化された認知機能検査を行った。検査はいずれもその標準的手順に従って実施およびスコア化するようにし、訓練を受けた神経心理士が担当した。各検査の公表された基準で標準化（T）スコアを算出した。特に関心のある3つの認知領域（記憶、実行機能、処理速度）および総合的認知機能のそれぞれについて、平均総合Tスコアを算出した。検査に用いた項目は、記憶（Hopkins Verbal Learning Test-Delay, Rey Complex Figure Test-Long Delay）、実行（Digit Span Backwards, Trail Making Test B, Wisconsin Card Sorting Total errors）またはStroop Color and Word Test-interferenceスコア、処理速度（Wechsler Adult Intelligence Scale—Digit Symbol and Symbol Searchのサブテスト2つ）である。総合評価項目はこれらの3項目と注意（Digit Span ForwardおよびTrial Making Test A）および言語[Boston Naming 60 item test, Controlled Oral Word Association（FAS）]の平均値が含まれていた。

MRI検査

検査はSiemens 1.5T Sonata MRI装置で開始したが、現在はSiemens 3T Trioにて行っている。BD患者の14例は1.5T MRIで2回撮像し、8例は3.0Tで撮像した。2回目の撮像は、すべて最初の撮像と同じ装置を使用し、測定に使用したのは構造をみるT1画像、WMHの特徴を知るためのFLAIR画像、BBB透過性を計算するためのpartial inversion recoveryによるT1マッピング（T1 mapping with partial inversion recovery; TAPIR）画像である。TAPIRは、ガドリニウムエチレントリアミンベンターサセット（Magnevist, Bayer Schering Pharma）注射後に撮像した1回目の連続、高速撮像、軸位断T1画像測定と注射後の複数のT1画像測定を使用する。ガドリニウムエチレントリアミンベンターサセットは標準用量の1/4量を自動注射器（Medrad Spectris SolarisVR MR Injection System, Siemens）で注射する。撮像パラメータは2つの装置の磁場強度でほぼ同じとしたが、3Tはやや解像度がやや高く、TAPIR画像も時間分解能が高かった。撮像パラメータをオンラインデータを用いた表に要約した。

構造画像解析

半自動ソフトウェアパッケージ（JIM V.6.0, Xinapse Systems Ltd, Northants, UK, http://www.xinapse.com）により、FLAIR画像のWMHを区別した。臨床情報が認められる3名の経験豊富な医師が半定量的FazekasスケールでWMHを評価し、同じ評価者がTI, T2およびFLAIR画像のそれぞれで血管周囲囊の評価およびラクナと皮質梗塞の計数を行った。

<table>
<thead>
<tr>
<th>特徴</th>
<th>BD＝22</th>
<th>対照＝12</th>
<th>P 値</th>
</tr>
</thead>
<tbody>
<tr>
<td>年齢、歳</td>
<td>67 ± 10</td>
<td>61 ± 9.5</td>
<td>有差なし</td>
</tr>
<tr>
<td>性別（男性）</td>
<td>41%</td>
<td>44%</td>
<td>有差なし</td>
</tr>
<tr>
<td>高血圧</td>
<td>86%</td>
<td>19%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>糖尿病</td>
<td>27%</td>
<td>0%</td>
<td>0.03</td>
</tr>
<tr>
<td>局所神経学的微候</td>
<td>68%</td>
<td>13%</td>
<td>0.001</td>
</tr>
<tr>
<td>静脈中の既往</td>
<td>50%</td>
<td>0%</td>
<td>0.001</td>
</tr>
<tr>
<td>運動失調／平衡失調</td>
<td>73%</td>
<td>6%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MRIの特徴</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ラクナ</td>
<td>82%</td>
<td>0%</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>WMHs</td>
<td>39.472 ± 2.474</td>
<td>702 ± 449</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fazekasスコア</td>
<td>5.2 ± 0.88</td>
<td>1.46 ± 0.77</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>血管周囲囊</td>
<td>5.3 ± 0.95</td>
<td>3.28 ± 1.13</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CSF／アルプミン比</td>
<td>7.16 ± 2.6</td>
<td>5.6 ± 2.8</td>
<td>0.04</td>
</tr>
</tbody>
</table>

認知機能評価

<table>
<thead>
<tr>
<th>項目</th>
<th>総合スコア</th>
<th>52.73 ± 8.9</th>
<th>0.02</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXE</td>
<td>43.86 ± 8.2</td>
<td>50.6 ± 5.9</td>
<td>0.01</td>
</tr>
<tr>
<td>MEM</td>
<td>46.5 ± 11.6</td>
<td>53.1 ± 8.9</td>
<td>0.07</td>
</tr>
<tr>
<td>SPEED</td>
<td>48.29 ± 10.4</td>
<td>55.6 ± 9.3</td>
<td>0.03</td>
</tr>
<tr>
<td>BS</td>
<td>5.8 ± 1.7</td>
<td>1.2 ± 1.8</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

BD：Binswanger病、BS：Binswangerスコア、CSF：脳脊髄液、EXE：実行機能、MEM：記憶、MRI：磁気共鳴情報、SPEED：処理速度、WMH：白質信号変化。

*Binswanger病の可能性78%となる。
来院時のWMPマップと来院2回目に新しく出現したWMAで、ボクセルの重なりを計算した（来院2回目のWMA－来院1回目のWMA）。この重なりは、前回からの透過性が異常であった新しいWMAボクセルの割合として報告した。

来院1回目と2回目のデータを合わせて、WMPの空間分布とWMAの関係を検討した。これを実施したのは、透過性マップの目目で透過性亢進領域がWMAの境界に集中していたためである。この解析では白質を（1）NAWM、（2）WMAの境界両側で2 mmずつWMAマスクを拡大および縮小することにより作成したWMA境界線の周縁4 mm、（3）周縁領域内のWMAの3つの関心領域に分類した。これら領域のそれぞれについてWMPを計算した。

統計
正規分布データを群間比較するにあたり、各変数の長期変化についてはSattertwaite Student t検定および対応のあるt検定を使用した。二項データの群間比較にあたっては、Fisher検定およびANOVAにより反復共変量分析を実施した。統計解析はSAS v12.1およびSPSS v20.0にて実施した。

結果
選択および除外基準を満たし、かつBDの臨床的特徴を認め22例および年齢の一致する16例が、2回の来院で脳画像検査および神経心理学的検査を完了した。患者と対照のベースライン時の特徴を表に示す。BDの被験者はすべて、2回の来院のいずれにおいても対照に

透過性解析
先に述べたように、Patlakのガドリニウム造影剤BBB漏出モデルに基づいて、TAPIRの測定値からボクセル単位の透過性マップを計算した。来院毎のT1画像、FLAIR画像、透過性マップと初回来院時のFLAIR画像の空間的配置を合わせ、線形化変換を評価した。透過性はWMAおよびNAWMの領域内のみ計算した。

白質はBrain Software Library（FSL、www.fmrib.ox.ac.uk/fsl/）のfast/Functional MRIによりT1画像を区切り決定し、白質部分を7 mmずつ削って灰白質および脳液との近接を避けた。この方法により、白質の透過性計算がより一貫するようになった。活発なBBB漏出領域は、透過性閾値0.003/minを超える領域と定義した。透過性閾値の範囲は0.001 ～ 0.005/minとし、0.003/minで対照と患者を正確に予測する精度が最大となった。症例数が少なかったため、この閾値はデータそのものから得た。WMPは活発なBBB漏出領域の透過性ボクセルの合計である。WMPはカットオフ閾値に依存する。

WMPと新しいWMAの関係を理解するため、初回
代表的歯是患者4例の透過性マップ。正常歯照は、主に歯室周囲に白血病性炎熱（WMH）の領域がいくつかある（矢印）。歯はWMH。赤は分散した透過性亢進領域、Binswanger病の歯は少しなかった。歯照では強い透過性亢進領域（黄色）が強く認められなかった。赤から黄色への色分けて透過性の亢進度を示した。

図3に年齢の一致する対照の歯例のWMHマップを示す。対照歯歯歯室周囲に小さなWMH斑がたまにみられた。

新たに発生したWMHと歯歯1回目のWMHマップを重ねると、新しいWMHボクセルの11%に前々から透過性異常が存在していた。同じように、透過性異常のあるWMHボクセルは14%であった。来院1回目のWMHマップを歯歯2回目と比較した。歯歯1回目で透過性が認めた領域のほとんどは来院2回目で消失し、1回目と2回目で重なったWMH亢進ボクセルは5±2%のみであった。来院1回目に透過性が亢進していた領域は1色で、来院2回目の領域は別の色で表示し、重なる領域は3番目の色で表示した（図4）。

来院1回目と2回目の画像でWMH領域を目視確認したところ、WMHの歯の辺りに透過性亢進が集中していた。透過性のある場所とWMHの関係を説明するため、WMHの境界線をトレースした4mmの周縁からなる関心領域を新たに抽出した。例として、FLAIR画像で

比べ合計WMHが高値であった（ANOVA、P=0.01）。患者のWMHは対照より有意に大きく分散していた（P=0.002、図1）。観察間隔の中央値は16.5ヵ月であった（四分位範囲12.5～22.6）。観察の間の数ヵ月を共変量とした反復測定によるWMHの変化は認められなかった（ANOVA、P=0.74）。来院1回目と2回目のWMHに個人間変動はみられたが、群内での変動は認められなかった（対応のあるt検定、P=0.22）。2回目の脳MRI検査で、WMH量（対応のあるt検定、P＜0.01）、Fazekasスコア（対応のあるt検定、P＜0.01）、血管周囲腔の数（対応のあるt検定、P＜0.01）は1回目より有意に増加した。観察内でのWMHとWMH量に関連が見出されなかった（ANOVA、P=0.22、図2）。来院1回目と2回目で無症候性ラクナ梗塞の有意な増加は認められなかった（対応のあるt検定、P=0.07）。時間経過とともにWMHおよび神経心理学的検査が悪化することとはなかった（データ非表示）。いずれの来院においても、BD患者の白質のどこかに透過性が異常亢進した領域が必ず認められた。

対照観察例にはほとんど透過性が認められず、認められた場合は白質でランダムに透過性が散らばるパターンを示した。
WMHの周りを縦取り、透過性を重ねたBD患者を示す（図5A）。WMHの一箇所に4 mmの周縁を描き、透過性を赤で表示した（図5B）。周縁の大きさの選択肢を選示するため、各被験者のプロットを示す。指数関数の傾斜から、4 mmの周縁領域に高透過性ポルセが集まっていることが分かる。高透過性ポルセは WMHの周縁領域に集中していた（図5C）。3.0 テスタの患者のみで作成した透過性マップを使用すると、合計した透過性ポルセの51%は4 mmの周縁領域内部にあり、9%はWMHコア内にあり、49%はNWM内にあった（ANOVA, P < 0.001）。

考 察

BD患者のBBBでは透過性の亢進が1～2年後まで持続することが分かった。1回目の画像でBBBの透過性が異常な部位は2回目の画像で消失する傾向にあったが、新たな領域が出現した。WMHの容積とWMFの相関は認められなかった。2つの画像でポルセ透過性マップを比較したが、ほとんど重なる部分はなく、BBBの破綻パターンは持続性と変動性の両方を持つと考えられた。BD患者のWMFは大きく変動したのに対し、対照のWMFはあまり変動しなかったことは、これらの変化が誤りでないことの証であり、WMFの変動が生物学的過程から生じることを示唆する。

本研究に独特な点は、透過性亢進領域でポルセマッピングで追跡する能力である。初期の透過性と新しいWMHはほとんど重ならなかったため、最終のWMF画像から将来のWMF形成を予測することはできなかった。また、2画像の透過性領域は殆ど重ならなかった。元々あった透過性亢進領域は時間経過とともに消失したが、新たな漏出領域が別の場合に出現した。最初のWMFマップと2回目の画像のわずかな重なりは、この透過性のバラツキによって説明がつくかもしれない。

このような透過性の空間的変動は、位置合わせの誤差、空間解像度の低さ、画像法の再現性などに起因する部分がある可能性があるため、各個人の画像のWMHとBBB透過性マップで空間的関連性を検討した。WMHの境界線の重ねをトレースしたことにより、ほとんどの高透過性ポルセがNAWMかWMHの周縁領域の中にあること、WMHコアの中にはわずかしかポルセが存在しないことが明らかになった。この研究の結果は、FLAIRで得たWMHの境界線が白質の絶対透過性変化に影響しないことを強く示唆する。代わりに、WMF亢進領域が WMHコアの外側からNAWM領域へと徐々に移動しているようである。この所見は疾患の進行を理解する上で特に注目される。白質の微細構造を測定するMRI方法、異方性（FA）を利用した研究で、FAが低下したベナックス領域はWMHを取り囲んでいた13。長期の追跡調査では、ベースラインのFA異常が将来のWMH形成を予測することが示された14、15。このようなFA変化はNAWM領域より拡大するWMF領域内で大きい16。白質のBBB破綻がWMFの周辺に集中するという本研究の結果は、局所のWMF拡大に関連する疾患活動度を表す別の指標になる可能性がある。白質のBBB障害は、内皮細胞の機能障害およびそれに続く白質変異を予測させる初期の現象かもしれない。脳卒中発症性高血圧ラットモデルで、この白質における初期のBBB破綻が皮質下虚血性変化の出現前に観察されている17。

ラクナ梗塞はSVDのもう一つの形態で、全虚血性脳卒中の20％を占め、WMHの存在と強く関連する18。
FLAIR で可視される WMH をとりまく NAWM が、将来の WMH 拡大領域および新しいラクナ梗塞の発生領域と一致することを示すエビデンスが増加している。これは SVD の遺伝型である皮質下梗塞および白質脳症を伴う常染色体優性遺伝性脳動脈症 (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; CADASIL) の患者でも認められている 18。WMH の境界に発生する新規の小さい散在強調画像病変が WMH の拡大に関与するという説もある 19。BBB の破綻はラクナ梗塞患者で認められてきたが、新たなラクナ梗塞発生の前触れか否かは不明である 20。BD 患者のラクナ梗塞は、脳画像検査によって偶然検出されることが多い。しかし WMH がなくても、ラクナ梗塞は頭蓋内のアテローム性ブラックや微小塞栓などの病態生理学的機序により発生する 21。このような場合、早期の BBB 破綻の関与は少ないであろう。したがってラクナ梗塞の病態生理学的機序は異なる可能性がある。DCEMRI および透過性マップを使用すれば、今後の研究でラクナ梗塞の病態生理学的機序を区別できるかもしれない。

本研究の発症の WMH は大い、これらの病変はオリゴソースを表すが、透過性の変化による炎症がすでに止まったということかもしない。WMH が大きい患者の NAWM では DCEMRI も FA も異常であるため、将来は介入試験の画像パラメーター候補としてこれら 2 つの方法の併用を評価する必要がある。

また、WMH からの動き NAWM で WM の亢進が認められた。他の研究グループが、BBB の画像負荷前の MRI 画像法を使用し、さまざまな SVD 患者群の NAWM で WMH 免量と BBB の透過性亢進に関連があることを明らかにしている 22。本研究の結果もこれと一致するが、WMH 免量と WM の関連を明らかにすることはできなかった。本研究は、WMH は運動性の疾患であり、WMH 免量は蓄積過程である、という考えを支持する。

NAWM の BBB が変化したこと、WMH の周囲で高透過性ポルクルの密度が上昇したことから、透過性が亢進した NAWM 領域は WMH の拡大しやすい領域であると推測される。さらに BD 患者の白質で、NAWM と WMH の周縁領域の付近で病理的 BBB 変化が起こったが、これは病理的過程がびまん性であることを示唆する。本研究の強みは WM その他の局所的可視化を初めて実現させたことである。注意しなければならない点は、症例数の少なさ、後方視デザイン、装置の精度の差、最適ではない空間分解能、割検による診断の検証がなかったことなどである。

以上、本研究は BD 患者で持続的な BBB 破綻が認められ、時間とともに白質領域内で変化することを明らかにした。WMH 周辺領域でみられた高透過性ポルクル数の増加は BBB 破綻と WMH 形成の関連性を強く示唆するものであるが、確認のため今後の研究が必要である。

研究費財源
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情報開示
なし。

References


빈스방거병에서 혈관뇌장벽(blood–brain barrier, BBB) 투과도의 장기간 변화

Long-Term Blood–Brain Barrier Permeability Changes in Binswanger Disease

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(Stroke. 2015;46:2413-2418.)

Key Words: blood–brain barrier ■ leukoaraiosis ■ magnetic resonance imaging ■ permeability ■ white matter

배경과 목적

알공(lacunes)과 백색질고음영(white matter hyperintensities, WMHs)이 있는 소혈관질환 환자에서는 혈관뇌장벽(blood–brain barrier, BBB)이 파괴된다. WMHs과 국소 BBB 투과도 변화의 관계는 지금까지 연구되지 않았다. BBB 파괴는 정상적으로 보이는 WM와 WMHs 주변부에서 일어난다는 가설을 세웠다. 가설을 시험하기 위해, 빈스방거병과 연관된 광범위한 WMHs가 있는 환자에서 BBB 투과도 측정을 반복하였다.

방법

잘 특정화된 대규모 전향적 혈관인지장애 코호트에서 22명의 빈스방거병 환자로 구성된 부분집합을 선택하였다. 비교를 위해 16명의 연령이 맞는 대조군을 사용하였다. 비정상 백색질투과도 (white matter permeability, WMP)는 역동적 조영증강 자기공명영상(dynamic contrast–enhanced MRI)을 사용하여 수년간 두 차례 측정하였다. WMP 지도는 미리 정해진 역지 이상의 복셀 (voxel)로부터 구축되었다. 첫 번째 및 두 번째 방문의 측정은 함께 동등하였다. WM은 3개 부분으로 나뉘었다: 정상으로 보이는 WM, WMH 주변부(ring), WMH 핵심부(core). 주변부는 WMH 경계의 양측 2 mm로 정의하였다. WMP는 3군데 특정 구역 각각에서 계산하였다. 우리는 개별 변화를 비교하기 위해 대응표본 t 검정, ANOVA, Fisher 정확검정을 사용하였다.
결과
WMP는 대조군보다 환자군에서 유의하게 높았다 (P<0.001). WMH 부담과 WMP 사이에는 상관관계가 없었다. 높은 투과도 영역은 첫 번째와 두 번째 촬영에서 약간 중첩되었다. WMP의 9%는 WMHs 내부였고, 49%는 정상으로 보이는 WM에, 52%는 WMH 주변부에 있었다 (P<0.001; ANOVA).

결론
정상으로 보이는 WM와 WMH 경계 가까이에서 증가된 BBB 투과도는 BBB 파괴와 WMHs 발생 간의 관련성을 지지한다.

Figure 1. White matter permeability (WMP) from visit 1, visit 2, and controls. There are significant differences in WMP between controls versusBinswanger disease (BD) subjects’ first and second visits (ANOVA, P<0.001), but no difference between patient visits. Box plots are shown with outliers as circles. WMP variances were higher in BD subjects than in controls (ANOVA, P=0.01).

Figure 3. Permeability maps in 4 representative control patients. The normal controls had some areas of white matter hyperintensities (WMHs) mainly in the periventricular regions (arrows). Green delineates the WMHs. The red areas of scattered increased permeability were lower than found in the Binswanger disease patients. None of the controls had regions of high permeability (yellow). The color code used showed increasing permeability from red to yellow.

급성혈혈뇌졸중에서 총 호모시스테인 수치의 상승은 장기간 사망과 연관된다

Elevated Total Homocysteine Levels in Acute Ischemic Stroke Are Associated With Long-Term Mortality

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(Stroke. 2015;46:2419-2425.)

Key Words: acute ischemic stroke ■ atherosclerosis ■ homocysteine ■ long-term mortality ■ stroke

배경과 목적
총 호모시스테인 수치는 이차적인 혈관질환의 발생 및 사망과 연관된다. 이 연구는 급성뇌졸중 시기에 총 호모시스테인 수치가 심뇌혈관질환의 재발 및 사망에 기여하는지를 조사하고자 하였다.