Decrease in Cortical Benzodiazepine Receptors in Symptomatic Patients With Leukoaraiosis
A Positron Emission Tomography Study

M. Ihara, MD, PhD; H. Tomimoto, MD, PhD; K. Ishizu, MD, PhD; T. Mukai, PhD; H. Yoshida, MD; N. Sawamoto, MD, PhD; M. Inoue, MD; T. Doi, MD; K. Hashikawa, MD, PhD; J. Konishi, MD, PhD; H. Shibasaki, MD, PhD; H. Fukuyama, MD, PhD

Background and Purpose—[11 C]flumazenil (FMZ), a ligand that selectively binds to the central benzodiazepine receptor in the neuronal membrane, is useful for evaluating neuronal viability in a positron emission tomography (PET) scan. Using this ligand, we investigated whether there was a correlation between neuronal integrity in various brain structures and dementia in patients with leukoaraiosis.

Methods—Twelve patients with extensive leukoaraiosis on magnetic resonance imaging were divided into groups of patients with or without dementia. Based on a 2-compartment, 2-parameter model that included metabolite-corrected arterial input and PET-measured cerebral radioactivity, the distribution volume of FMZ (FMZ-Vd) was calculated in various regions of interest by nonlinear curve fitting. Additionally, tracer kinetic analysis was applied for voxel-by-voxel quantification of FMZ-Vd, and data analysis was performed by statistical parametric mapping.

Results—The presence of dementia was associated with a reduced FMZ-Vd in widespread areas of the cerebral cortex, including the bilateral frontopolar and frontal/insular areas, the left temporo-occipital border areas, and the left marginal cortical areas.

Conclusions—Differences in neuronal integrity in the cerebral cortex might determine whether patients with leukoaraiosis become symptomatic or not. (Stroke. 2004;35:942-947.)

Key Words: leukoaraiosis ▪ Binswanger’s disease ▪ receptors, benzodiazepine ▪ tomography, emission computed
dementia and those who did not by using the PET scan. As a tracer, we used the central benzodiazepine receptor (cBZR) ligand \([11\text{C}]\text{flumazenil} (FMZ)\), because coupling of cBZRs to \(\gamma\)-aminobutyric acid type A (GABA\(\text{A}\)) receptors, which are widely expressed in cerebral cortical neurons, makes FMZ a reliable marker of neuronal integrity.\(^{15,16}\) We also examined cerebral blood flow (CBF), cerebral metabolic rate of oxygen metabolism (CMRO\(\text{2}\)), and oxygen extraction fraction (OEF) by using the \([15\text{O}]\) gas steady-state method in these patients.

**Materials and Methods**

**Subjects**

Twelve patients whose T2-weighted MRI scans revealed confluent hyperintensities in the subcortical white matter (Schmidt scale score of 3)\(^{17}\) and several punctate high-intensity areas in the basal ganglia were studied by PET (Figure 1). They were enrolled in this study because of their MRI-proven neuroradiologic appearances after visiting our Neurology Clinic from May 1999 to March 2002 with various neurologic signs and symptoms. Patients with mild to moderate leukoaraiosis (Schmidt scale score of 1 or 2) were not enrolled in this study to exclude the possibility of leukoaraiosis associated with neurodegenerative disorders such as Alzheimer’s disease. Each subject was fully instructed on the experimental procedures and provided written, informed consent, as approved by the Committee of Medical Ethics of our faculty. If the subject was not fully competent due to dementia, we obtained full informed consent from a proxy. None of the patients had apparent lesions in the cerebral cortex or hippocampus. MR angiography or duplex color-coded sonography did not reveal \(>50\%\) stenosis in the major intracranial and extracranial vessels. No patient had a history of taking any drugs within the past 3 months that would affect BZR assessment. All subjects underwent a general physical and neurologic examination and neuropsychological assessment, including the Clinical Dementia Rating (CDR)\(^{18}\) and Mini-Mental State Examination (MMSE). At least 2 neurologists were involved in the neuropsychological assessment independently. If their assessments did not coincide, patients were reexamined by the team for a final verdict.

**Synthesis of \([11\text{C}]\) FMZ**

\([11\text{C}]\) FMZ was synthesized as previously described.\(^{19}\) The radiochemical purity of \([11\text{C}]\) FMZ was \(>99.0\%\), and the specific activity of the product was \(52.5\pm15.5\text{ GBq}/\text{mol} (n=12)\).

**PET Scan**

The subjects were scanned with a PET scanner (Advance, General Electric) in 2-dimensional mode for FMZ-PET, as previously reported.\(^{19}\) Arterial blood samples were drawn, and a metabolite correction of \([11\text{C}]\)FMZ was performed by the plasma extraction method.\(^{19}\) Dynamic imaging was performed in 2-dimensional acquisition mode for 50 minutes after injection (sequence: 6\times30\text{ seconds}, 7\times1\text{ minute}, 5\times2\text{ minutes}, and 6\times5\text{ minutes}).

All patients except 1 demented woman underwent a \([15\text{O}]\) gas steady-state study for quantitative CBF and CMRO\(\text{2}\) with the same PET scanner. We followed the protocol for inhalation of \([15\text{O}]\)CO\(_2\), \([15\text{O}]\)O\(_2\), and \([15\text{O}]\)CO as previously reported.\(^{20}\)

**Data Analysis**

For the analysis of PET images obtained under similar conditions, PET data were reconstructed into 3-dimensional images parallel to the orbitomeatal line, so that each image consisted of 64 planes with 2-mm cubic voxels. Images were displayed by using PMOD software version 2.4 (PMOD Group).\(^{21}\)

**ROI-Based Analysis**

Regions of interest (ROIs) were defined on the summed FMZ uptake images by point-and-click or manual drawing mode of PMOD (Figure 2A). Because there are few BZRs in the subcortical regions and none

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**Figure 1.** Representative T2-weighted MRIs and summed FMZ images in 2 patients with leukoaraiosis, 1 of whom manifested dementia (A) and the other of whom manifested no dementia (B). Although the MRIs from these 2 patients contain diffuse hyperintensities in the white matter (Schmidt scale 3) and multiple lacunas in the thalamus and basal ganglia, FMZ images show clear contrast in the cortical binding.

**Figure 2.** A, ROIs defined on the summed \([11\text{C}]\)FMZ uptake image. B–D, Comparisons of FMZ-\(V_e\) (B), CBF (C), and CMRO\(\text{2}\) (D) in the ROIs between demented (solid bars) and non-demented patients (open bars) with leukoaraiosis. Bars represent SEM. Significant differences (\(P<0.05\)) between groups are indicated by asterisks. oF indicates orbitofrontal cortex; pF, prefrontal cortex; T, temporal; P, parietal; O, occipital; Th, thalamus; BG, basal ganglia; Cb, cerebellum; and CS, centrum semiovale.
in the subdural cerebrospinal fluid space, we could define the cortical mantle, rich in BZRs, by using the differences in FMZ uptake among these regions. For cortical regions, ROIs were defined in the following regions: orbitofrontal cortex, including Brodmann areas 11 and 12; anterior and dorsolateral prefrontal cortex, including areas 9, 10 and 46; temporal cortex, including areas 21, 22, and 37; parietal cortex, including areas 5, 7, 39, and 40; and occipital cortex, including areas 17, 18, and 19, while avoiding intracranial sulci to minimize the partial-volume effect. We also defined ROIs in the thalamus, basal ganglia, cerebellum, and centrum semiovale. ROIs for the centrum semiovale were carefully drawn to prevent contamination by ROIs in the ventricles and gray matter. No ROIs were placed on the periventricular regions because these structures were not easily separable from the ventricles on the FMZ uptake image. Based on a 2-compartment, 2-parameter model with metabolite-corrected arterial input and PET-measured cerebral radioactivity, the distribution volume of FMZ (FMZ-Vd) was calculated in the defined ROIs by nonlinear curve fitting based on the Gauss-Newton method. Based on the 2-compartment model, the distribution volume of FMZ (FMZ-Vd) was calculated in the defined ROIs by nonlinear curve fitting based on the Gauss-Newton method.

Voxel-by-Voxel Analysis

Using Pmod, pixel-wise calculation was performed to yield parametric images of FMZ-Vd. Briefly, the loaded image data were first pre-processed with arterial input curve. Classic Logan plot model was then applied to the image vector in each individual pixel. The pixel-wise results were assembled into parametric images of FMZ-Vd. These parametric images were analyzed using SPM2 (Wellcome Department of Cognitive Neurology) implemented in Matlab 6.5 (The MathWorks Inc). The images were transformed into the standard SPM2 PET template using the early phase of FMZ image added (0 to 10 minutes) as a blood flow image. As a final pre-processing step, the images were smoothed using a 10×10×10 (full width at half maximum) isotropic Gaussian kernel.

Figure 3. Comparison of regional brain volumetry between demented and nondemented patients with leukoaraiosis. Brain atrophy was assessed on the 3 axial slices; immediately superior to the ventricle (A), through the body of the ventricle (B), and through the anterior and posterior ventricular horns (C). Regions occupied by brain parenchyma, subdural space, and ventricular space are indicated as the ratio to total intracranial area at each level.

Figure 4. Voxel-based analysis of [11C]FMZ-PET, demonstrating significant decreases in FMZ-Vd in the bilateral frontopolar areas, the bilateral frontal/insular areas, the left temporoparietal border areas, and the left marginal cortical areas in demented patients (Pcorr<0.001). Statistical results are overlaid onto the brain surface (A) and slices (B) of the standard MRI template of SPM2. Color scale: t scores (maximum t=6.34, corresponding to Z=3.78), R indicates right.

MRI

Brain MRIs were obtained with a 1.5-T MR scanner (Signa Horizon, General Electric). T1-weighted axial images were obtained with a spin-echo pulse sequence with a repetition time of 400 ms and an echo time of 15 ms. Axial T2-weighted images were also obtained (repetition time, 3000 ms; echo time, 100 ms). Axial images were obtained in parallel to the orbitomeatal line. Slice thickness was 5 mm, with an interslice gap of 1.8 mm in the axial plane.

The extent of cerebral atrophy was assessed by regional volumetric measures normalized for total intracranial area on 3 T2-weighted axial slices (see Figures 1 and 3) on a Macintosh PowerPC computer with the use of public domain NIH Image 1.61 software (National Institutes of Health, Bethesda, Md). In brief, we manually outlined the inner boundary of the calvarium on T2-weighted axial images to determine the total intracranial area. The images were then binarized with the intensity threshold set at 60% of mean intracranial pixel values within the outlined area. After the ventricular and subdural areas were semiautomatically outlined with the wand tool, the number of pixels in each area was divided by that in the total intracranial area to calculate normalized ventricular and subdural areas. Finally, subtraction of the normalized ventricular and subdural areas from the total intracranial area (value 1.0) yielded normalized parenchymal area.

Statistical Analysis

The statistical significance of intergroup differences was assessed with Fisher’s exact test for categorical variables, and the Mann-Whitney U test was used for continuous variables of demographic data and by ANOVA for regional brain volumetry and ROI-based analysis of FMZ-Vd, CBF, CMRO2, and OEF (StatView5.0, SAS Institute).
Demographic Features of the Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Leukoaraiosis With Dementia (BD)</th>
<th>Leukoaraiosis Without Dementia</th>
<th>P</th>
</tr>
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<tr>
<td>No. of patients</td>
<td>6</td>
<td>6</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean age (range), y</td>
<td>76.0±3.7 (69–80)</td>
<td>74.2±4.9 (68–80)</td>
<td>0.48</td>
</tr>
<tr>
<td>CDR, (No.)</td>
<td>1 (n=1), 2 (n=3), 3 (n=2)</td>
<td>0 (n=4), 0.5 (n=2)</td>
<td></td>
</tr>
<tr>
<td>Memory (0–3)</td>
<td>2.0±1.0</td>
<td>0.3±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Orientation (0–3)</td>
<td>2.0±1.0</td>
<td>0.1±0.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Judgement (0–3)</td>
<td>2.2±0.8</td>
<td>0.3±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Community affairs (0–3)</td>
<td>1.9±1.0</td>
<td>0.2±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Home and hobbies (0–3)</td>
<td>1.9±1.0</td>
<td>0.3±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Personal care (0–3)</td>
<td>1.7±0.8</td>
<td>0.0±0.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MMSE score (0–30)</td>
<td>18.6±3.7</td>
<td>29.0±1.3</td>
<td>&lt;0.01</td>
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<tr>
<td>Orientation (0–10)</td>
<td>6.6±1.8</td>
<td>9.7±0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Registration (0–3)</td>
<td>2.6±0.4</td>
<td>3.0±0.0</td>
<td>0.30</td>
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<tr>
<td>Attention and calculation (0–5)</td>
<td>2.2±1.6</td>
<td>5.0±0.0</td>
<td>&lt;0.01</td>
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<tr>
<td>Recall (0–3)</td>
<td>1.8±1.1</td>
<td>2.5±0.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Language (0–9)</td>
<td>5.6±1.5</td>
<td>8.8±0.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gait disturbance, No. (%)</td>
<td>5 (83)</td>
<td>2 (33)</td>
<td>0.24</td>
</tr>
<tr>
<td>Dysarthria, No. (%)</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Urinary incontinence, No. (%)</td>
<td>4 (67)</td>
<td>0 (0)</td>
<td>0.06</td>
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<tr>
<td>Hypertension, No. (%)</td>
<td>5 (83)</td>
<td>2 (33)</td>
<td>0.24</td>
</tr>
<tr>
<td>BP control (systolic BP &gt;140 mm Hg), No. (%)</td>
<td>1 (17)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Diabetes mellitus, No. (%)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Cigarette smoking, No. (%)</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Microalbuminuria, No. (%)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Previous cerebrovascular events, No. (%)</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Previous myocardial infarction, No. (%)</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Use of antiplatelets, No. (%)</td>
<td>5 (83)</td>
<td>3 (50)</td>
<td>0.55</td>
</tr>
<tr>
<td>Use of anticoagulants, No. (%)</td>
<td>1 (17)</td>
<td>1 (17)</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.72±0.64</td>
<td>5.78±1.06</td>
<td>0.81</td>
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<td>Triglyceride, mmol/L</td>
<td>1.51±0.70</td>
<td>1.22±0.58</td>
<td>0.63</td>
</tr>
<tr>
<td>Hematocrit, proportion of 1.0</td>
<td>0.37±0.03</td>
<td>0.37±0.03</td>
<td>0.87</td>
</tr>
<tr>
<td>Thrombin-antithrombin complex, μg/L</td>
<td>4.0±2.3</td>
<td>2.4±0.4</td>
<td>0.20</td>
</tr>
<tr>
<td>Fibrogen, μmol/L</td>
<td>9.79±1.18</td>
<td>9.38±3.12</td>
<td>0.83</td>
</tr>
<tr>
<td>No. of lacunas in the basal ganglia and thalamus</td>
<td>2.5±1.5</td>
<td>2.3±2.1</td>
<td>0.48</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD. BP indicates blood pressure.

For voxel-based analysis, significant differences in FMZ-V$_2$ between groups were estimated according to the general linear model at each and every voxel of the normalized and smoothed images. A linear contrast was used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constituted a statistical parametric map (SPM[t]). The SPM[t] was thresholded at $P_{uncorr}$<0.001 without multiple comparison.

Results

Patient clinical features and demographics are summarized in the Table. Based on their CDR scores, the subjects were divided into 2 groups: demented (group D: CDR of 1, 2, or 3; 1 man, 5 women) and the nondemented (group ND: CDR 0 or 0.5; 3 men, 3 women). There were no significant differences in patient age between the groups. Scores in all 6 cognitive-functional categories of CDR were significantly different between the 2 groups (the Table). Among the 5 cognitive-functional categories of the MMSE, scores in orientation, attention and calculation, and language were significantly different between the groups (the Table). All patients had at least 1 risk factor for ischemic cerebrovascular disease. Although there were no intergroup differences for these categorical variables, neurologic deficits were more frequently found in group D. All 6 demented patients met the criteria of NINDS-AIREN for vascular dementia. The mean±SD number of lacunas in the basal ganglia and thalamus did not differ between the 2 groups.

FMZ-V$_2$ was lower in group D than in group ND in all ROIs (Figure 2B). These reductions reached significance in the orbitofrontal cortex (11.2%), anterior/dorsolateral prefrontal cortex (13.4%), temporal cortex (14.1%), and parietal cortex (13.1%), although significance was not reached in the occipital cortex, thalamus, basal ganglia, cerebellum, and
centrum semiovalis. CBF and CMRO₂ were also reduced in group D (Figure 2C and 2D). The reduction in CBF reached significance in all cortices examined (orbitofrontal cortex, 20.1%; prefrontal cortex, 21.2%; temporal cortex, 20.7%; parietal cortex, 19.2%; and occipital cortex, 19.6%) as well as in the basal ganglia (26.2%). CMRO₂ was significantly reduced in the orbitofrontal cortex (21.3%), anterior/dorsolateral prefrontal cortex (23.5%), temporal cortex (20.3%), parietal cortex (18.2%), basal ganglia (28.1%), and centrum semiovalis (29.0%). No significant differences were detected in OEF.

SPM analysis showed that FMZ-Vₐ was significantly reduced in the bilateral frontopolar areas, the bilateral frontal/insular areas, the left temporo-occipital border areas, and the left marginal cortical areas (Pcorr < 0.001; Figure 4).

The regional volumetry showed no significant differences in brain parenchymal, ventricular, and subdural areas at the 3 axial levels between D and ND groups (Figure 3). The ventricular space was larger in group D, whereas the subdural space was larger in the ND group.

Discussion

The aforementioned results show that decreases in FMZ-Vₐ, CBF, and CMRO₂ are widespread in the cerebral cortices in patients with leukoaraiosis who manifest dementia (BD patients) compared with those who do not. Because no significant differences were detected in subdural space between groups D and ND, such difference in FMZ-Vₐ, CBF, and CMRO₂ cannot be attributed to partial-volume effects. FMZ binding reflects neuronal integrity and is relatively unaffected by CBF. Our results therefore suggest that neuronal integrity is impaired in BD patients in the cortices where CBF and oxidative metabolism are also decreased. Although the sex ratio was not completely matched between the 2 groups (there were more women in group D), adjustment for sex would likely have further widened the difference between the 2 groups because FMZ-Vₐ was reported to be slightly higher in women than in men. In contrast, MRI revealed no significant differences in the parenchymal space, the severity of white matter lesions, or the number of lacunas between demented and nondemented patients with leukoaraiosis. Therefore, neuronal damage that is not visible on conventional MRI might be present in the cerebral cortex and might contribute, at least partially, to the development of dementia.

Leukoaraiosis and fibrohyalinosis of the medullary arteries are predominant in the frontal lobe. In the present SPM analysis, the greatest reductions of FMZ-Vₐ were seen in the bilateral frontopolar and frontal/insular areas in demented patients with leukoaraiosis. Yao et al. and Sabri et al. reported that CBF and CMRO₂ were significantly reduced not only in the cerebral cortices but also in the basal ganglia. Because the frontal cortex and the basal ganglia constitute closely connected corticosubcortical circuits through the frontal white matter tracts, extensive leukoaraiosis in the frontal white matter might cause circuit disruption and thereby, a loss of executive function and processing speed. Furthermore, frontopolar and frontal/insular areas are postulated to participate in the active maintenance of attention during memory retrieval and other cognitive functions. Such top-down control of attention by the frontal cortex might be impaired and therefore contribute to the development of cognitive slowing in patients with leukoaraiosis. Consistent with this notion is that the attention and calculation category of the MMSE was most significantly decreased in demented patients with leukoaraiosis.

Leukoaraiosis seen on T2-weighted images might be due to a variety of different pathologic conditions. In fact, differences in the type and distribution of these lesions might account for differences in neurologic symptoms. Thus, oxidative metabolism in the deep white matter was significantly reduced in demented compared with nondemented patients, suggesting that different pathologies underlie apparently similar T2 hyperintensities in the white matter. More severe white matter damage, such as a loss of synaptophysin, might lead to neuronal disintegration as well as dementia. Because conventional MRI cannot differentiate underlying white matter pathologies, functional imaging including FMZ-PET can be an effective premortem modality to detect such circuit disruption. In fact, axonal damage has been reported in autopsied specimens of white matter lesions and in demented patients with leukoaraiosis who were examined by ¹H MR spectroscopy. Axonal damage can cause retrograde degeneration, which might result in neuronal perikaryal injury and, ultimately, neuronal loss. Indeed, loss of synaptophysin, a neuronal marker, in the cerebral cortex has been reported in the brains of BD patients. Our results are consistent with these findings and further suggest that neuronal injury in the cerebral cortex occurs in BD patients.

In summary, the current study suggests that severe ischemic damage in the white matter can evoke corticosubcortical circuit disruption, possibly leading to impaired top-down control by the frontal cortex. Although a longitudinal study is required to ascertain whether nondemented patients with leukoaraiosis will develop full-blown dementia in the natural course of the disease, therapeutic intervention might be effective to prevent cognitive impairment. Recent evidence suggests promising effects of several acetylcholine esterase inhibitors on symptomatic relief of vascular dementia, however, no definitive therapy has been developed. Therefore, further research is warranted to develop novel strategies to maintain the functional integrity of corticosubcortical circuits.

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References

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