Effect of Thrombin Inhibition in Vascular Dementia and Silent Cerebrovascular Disease
An MR Spectroscopy Study

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Background and Purpose—Silent cerebrovascular disease (CVD) has been proposed as a predisposing condition for clinically overt stroke and vascular dementia. Recently, we found increased thrombin generation in silent CVD patients. Here, we report the effect of thrombin inhibition using a potent selective thrombin inhibitor on the cerebral metabolism and function in peripheral arterial occlusive disease (PAOD) patients with or without silent CVD.

Methods—We examined 17 mild chronic PAOD patients, including 2 cases of vascular dementia. We divided the patients into 2 groups: 1 was the advanced CVD group with multiple lacunar infarction and/or advanced periventricular hyperintensity detected by brain MRI (n = 12), and the other was the no CVD group that had none of these abnormalities (n = 5). We assessed the cerebral biochemical compounds in the deep white matter area and cerebellar hemisphere (8 cm³) by proton MR spectroscopy before and after infusion of argatroban (10 mg/d IV) over 2 hours for 7 days.

Results—The ratio of N-acetylaspartate (NAA) to total creatine (Cre) in the deep white matter area was significantly lower in the advanced CVD group than in the no CVD group, whereas there were no significant differences in this ratio in the cerebellar hemisphere between the 2 groups. In the former group, this decreased NAA/Cre ratio significantly increased after argatroban therapy, whereas there was no change in the latter group. The 2 patients with vascular dementia showed clinical improvement with marked increases in the NAA/Cre ratio and mini-mental score.

Conclusions—These results suggest that increased thrombin generation may have some pathophysiological roles in developing vascular dementia and its chronic predisposing conditions. Thrombin inhibition may break this vicious cycle and lead to clinical improvement. (Stroke. 1999;30:1033-1037.)

Key Words: cerebral metabolism | cerebrovascular disorders | dementia, vascular | spectroscopy, nuclear magnetic resonance | thrombin

Silent lacunar infarction and advanced periventricular hyperintensity (PVH) detected by brain MR imaging, often found in the elderly with hypertension, have been proposed as predisposing conditions for clinically overt stroke and vascular dementia. The risk factors related to this condition have not been extensively studied. Thrombin, a thrombogenic serine protease, is activated from prothrombin in the circulation at the site of cellular injury, initiating the coagulation cascade. Recent studies have demonstrated that thrombin also regulates a number of important activities in cells of the central nervous system that have been found to have thrombin receptors and that thrombin activity not only is a factor in acute central nervous system injury but also may contribute to such neurodegenerative disorders as Alzheimer’s disease. Thrombin retracts neurites on cultured neuroblastoma cells and human fetal neurons and induces apoptosis; however, a very low dose of thrombin may have some protective action.

We have recently found that the plasma levels of prothrombin fragment 1+2 (F1+2), a sensitive marker of in vivo thrombin generation, were higher in the asymptomatic patients with lacunar infarction (especially multiple lacunar infarction) and/or advanced PVH. This suggests the involvement of thrombin in the pathogenesis of these predisposing conditions for vascular dementia. We speculated that the inhibition of thrombin leads to improvement in vascular dementia and its subclinical conditions. In this article, we report the effects of thrombin inhibition using the potent selective thrombin inhibitor argatroban on the cerebral biochemical states in 17 chronic peripheral arterial occlusive disease (PAOD) patients, including 2 cases of vascular dementia.
Subjects and Methods

Subjects
We studied 17 patients with mild chronic PAOD of the Fontain classification I or II. All patients were neurologically normal, except for 2 patients with vascular dementia. We divided these patients into 2 groups: 1 was the advanced cerebrovascular disease group having ≥3 silent lacunae defined as a low-signal-intensity area (>3 mm and <1 cm) on T1-weighted images and hyperintense lesions on T2-weighted images and/or advanced PVH on T2-weighted images by 1.5-T MRI imaging (Signa 5.4, GE Medical Systems; n=12), and the other group was the no cerebrovascular disease group having none of these abnormalities (n=5).

PVH on T2-weighted images was classified into the following 4 groups. Grade 1 PVH was defined as no abnormality or minimal periventricular signal hyperintensities in the form of caps confined exclusively to the anterior horns or rims lining the ventricle, grade 2 as caps in both the anterior and posterior horns of lateral ventricles or periventricular unifocal patches, and grade 3 as multiple periventricular hyperintense punctate lesions and their early confluent stages. Multiple areas of high signal intensity that reached confluence in the periventricular region were defined as grade 4. Because only 1 patient showed grade 4 PVH, this patient and those with grade 3 were considered to have advanced PVH. All MR images were interpreted under blind conditions by 2 authors.

There were no significant differences in characteristics between the 2 groups (mean±SD age, 72±6.4 versus 67±8.0 years; male, 42% versus 40%; hypertension, 50% versus 60%; hyperlipidemia, 50% versus 40%; diabetes mellitus, 33% versus 40%; smoking, 33% versus 40%; receiving antplatelet therapy, 100% versus 100%; former versus latter group). Informed consent was obtained from each patient or from the family members of the 2 patients with vascular dementia.

MR Spectroscopy
Proton (hydrogen-1) MR spectroscopy (H-1 MRS) was performed in the deep white matter and cerebellar hemisphere before and after infusion of argatroban (10 mg/d IV) over 2 hours for 7 days. Double-spin echo localization and selective excitation/homospoil water suppression were used (echo time, 136 ms; repetition time, 2000 ms; 128 scans; 8-cm3 voxels).10 Voxels were precisely located in the same areas (defined by the horizontal and coronal sections) of the periventricular deep white matter and cerebellar hemisphere (Figure 1). An automated sequence—which set the radiofrequency pulse power, optimized the magnetic field homogeneity, and adjusted the water suppression—was used. The total imaging and spectroscopy scan time was 40 minutes. A Sparcstation 10/30 workstation (Sun Microsystems) was used for spectral data analysis with SAGE/IDL software (GE Medical System). After exponential filtering, zero filling, and Fourier transformation, the peaks for water, N-acetylasparate (NAA), total creatine (Cre), choline-containing compounds (Cho), and mioinositol (MI) were fitted by use of the Marquardt fitting algorithm, and the coefficients of variation for any spectra (Figure 1). The NAA/Cre ratio in the deep white matter area was significantly lower in patients with vascular dementia than in those with no cerebrovascular disease group and the no cerebrovascular disease group, and those 2 thrombin generation markers were significantly lower after argatroban therapy (Table 1). Clinical signs or symptoms related to PAOD (cold feeling of skin, intermittent claudication, or cyanosis) were improved in 12 (71%) of the 17 patients.

Blood Sampling and Assay Procedure
After a minimum fasting period of 12 hours, blood samples were collected in disposable siliconized vacuum glass tubes containing 3.8% trisodium citrate (9 volumes of blood to 1 volume of 0.13 mol/L trisodium citrate solution), and blood samples in the second tube were used. Samples were centrifuged at 3000 g for 15 minutes at room temperature within 1 hour of collection. Plasma was subsequently separated and stored in plastic tubes at −80°C until laboratory determinations were performed.

The plasma antithrombin III (ATIII) level was determined by a chromogenic assay with the use of Berichrom ATIII (Behringwerke AG). We measured plasma levels of molecular markers of thrombin generation (thrombin-ATIII [TAT] and F1+2) and d-dimer using ELISA kits for TAT (Teijin Co Ltd), F1+2 (Behringwerke AG), and d-dimer (Diagnostica Stago). The coefficients of variation for analytical assays were 2.5% for ATIII, 5.5% for TAT, 3.6% for F1+2, and 3.8% for d-dimer.

Statistical Analysis
Data are shown as mean±SD or percentage. Welch’s t test was used to compare the mean values in the 2 groups, and mean values in samples from the same patient were compared by the paired t test, with P<0.05 indicating statistical significance.

Results
Molecular Markers of In Vivo Thrombin Generation
There were no significant differences in baseline plasma levels of molecular markers of in vivo thrombin generation (TAT and F1+2), d-dimer, or ATIII between the advanced cerebrovascular disease group and the no cerebrovascular disease group, and those 2 thrombin generation markers were significantly lower after argatroban therapy (Table 1). Clinical signs or symptoms related to PAOD (cold feeling of skin, intermittent claudication, or cyanosis) were improved in 12 (71%) of the 17 patients.

Cerebral Metabolisms Assessed by H-1 MRS
H-1 MRS revealed 4 peaks: NAA at 2.0 ppm, Cre at 3.0 ppm, Cho at 3.2 ppm, and MI at 3.6 ppm; lactate was not detected in any spectra (Figure 2). The NAA/Cre ratio in the deep white matter area was significantly lower in patients with advanced cerebrovascular disease than in those with no cerebrovascular disease (P<0.05) (Table 2 and Figure 3). However, there were no significant differences in this ratio in the cerebellar hemisphere between the 2 groups. Other ratios, such as Cho/Cre and MI/Cre in areas of both deep white
Cerebral Function and Metabolism in Vascular Dementia

In the 17 patients with chronic PAOD studied, there were 2 cases with concomitant vascular dementia (Figure 3). One patient was a 77-year-old man, and 1 year ago, his family noticed that his intellectual ability had decreased. On examination, the neurologist diagnosed vascular dementia. Two months ago, he noticed intermittent claudication and resting paresthesia in his left leg; he was administered intermittent argatroban (10 mg/d IV) for 21 days. His frequent meaningless walking at night (3 to 7 times per night) disappeared completely during and after argatroban therapy. His intellectual ability assessed by the mini-mental score improved from 13 to 22 (full score, 30). H1-MRS disclosed a gradual increase in the NAA/Cre ratio after argatroban therapy (1.27 on the first test and 1.77 on day 21 after argatroban therapy; Figure 2).

The other case was a 69-year-old women who had intermittent claudication and vascular dementia (as diagnosed by a neurologist) for 8 months. After her diagnosis, we administered intermittent argatroban (10 mg/d IV) for 7 days. Her intellectual ability assessed by the mini-mental score improved from 13 to 21 on day 7 after argatroban therapy; 1.59 on day 7 after argatroban therapy; Figure 2).

Discussion

In the present study, we first demonstrated that systemic thrombin inhibition could improve the impaired cerebral metabolism in patients with silent cerebrovascular disease. Furthermore, this improvement was accompanied by clinical improvement in patients with vascular dementia.

For this study of thrombin inhibition in vascular dementia and its predisposing disease (silent cerebrovascular disease), we used argatroban, a potent selective thrombin inhibitor, and studied patients with PAOD because the indication of argatroban is accepted only for acute cerebral infarction and PAOD in Japan. The TAT and F1+2 levels (significantly higher in the PAOD patients before argatroban therapy than in age- and sex-matched control subjects) were significantly lower after argatroban therapy, suggesting that argatroban therapy (10 mg/d) suppresses thrombin generation in vivo.
These results are consistent with our previous findings in a multicenter clinical trial that used argatroban on PAOD patients.\textsuperscript{11}

Concerning the cerebral metabolism assessed by H-1 MRS, the NAA/Cre ratio of the deep white matter area was significantly lower in the advanced cerebrovascular disease group than in the no cerebrovascular disease group. NAA is located almost entirely in neurons and axons, and it is reduced in several cerebral diseases.\textsuperscript{10,12–14} In addition, its reduction correlates with clinical variables in patients after stroke.\textsuperscript{14} Thus, the NAA/Cre ratio is used to indicate neuronal injury or death. A recent report disclosed that this ratio was lower in symptomatic patients with advanced PVH.\textsuperscript{10} Thus, considering that there were no significant differences in this ratio in the cerebellar hemisphere between the 2 groups and that other ratios, such as the Cho/Cre and MI/Cre ratios in areas of both deep white matter and cerebellar hemisphere, were not significantly different between the 2 groups, a decreased NAA/Cre ratio in the deep white matter area with advanced cerebrovascular disease would indicate neuronal damage.

In the advanced cerebrovascular disease group, the low NAA/Cre ratio was significantly increased after argatroban therapy. In addition, after argatroban therapy, scores of mini-mental state assessment improved, along with clinical improvement in activity of daily living in patients with vascular dementia. The mechanism of this increase in the NAA/Cre ratio and rapid clinical improvement after argatroban therapy remains unclear. In advanced cerebrovascular disease patients with multiple lacunar infarctions or advanced PVH in whom the focal blood-brain barrier is destroyed, intravascular thrombin generation might easily transudate to the central nervous system, leading to further adverse effects on the neurofunctional status. Under this condition, the low-molecular-weight thrombin inhibitor argatroban may also easily pass the blood-brain barrier, inhibit focal thrombin around the neurons, and increase the NAA/Cre ratio, resulting in clinical improvement in vascular dementia patients. This rapid clinical improvement might be dependent on reversible neuronal dysfunction without neuronal death. The NAA/Cre ratio might be an indicator of reversible neurofunctional status in vascular dementia.

On the other hand, there was no change in the NAA/Cre ratio after argatroban therapy in the no cerebrovascular disease group. In healthy subjects, intravascular thrombin generation did not directly affect neurons because of the blood-brain barrier in cerebral arteries. Argatroban also could not penetrate the normal blood-brain barriers. Furthermore, the NAA/Cre ratio in the no cerebrovascular disease group was within the normal reference value, so no additional increase would be found after therapy.

The reason that the changes in the NAA/Cre ratio after argatroban therapy did not correlate with the changes in symptoms and signs of PAOD or with changes in the markers of thrombin generation remains unclear, although the NAA/Cre ratio symptoms and signs of PAOD thrombin generation markers were all essentially improved. In a previous study on PAOD patients, we found clinical improvement not only in patients with high TAT levels but also in those with low TAT levels. In this study, we confirmed these results, suggesting that local thrombin generation, which is closely related to clinical symptoms of PAOD, is not reflective of an increase in the amount of thrombin generation in the bloodstream (TAT

<table>
<thead>
<tr>
<th>Cerebrovascular Disease</th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>After</th>
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<tr>
<td>Deep white matter</td>
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<td></td>
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<tr>
<td>NAA/Cre ratio</td>
<td>1.45±0.13</td>
<td>1.43±0.13</td>
<td>1.34±0.08*</td>
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<tr>
<td>Cho/Cre ratio</td>
<td>0.96±0.15</td>
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<td>MI/Cre ratio</td>
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<td>0.60±0.15</td>
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<tr>
<td>Cerebellar hemisphere</td>
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<tr>
<td>NAA/Cre ratio</td>
<td>0.99±0.15</td>
<td>0.88±0.13</td>
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<tr>
<td>MI/Cre ratio</td>
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<td>0.49±0.15</td>
<td>0.51±0.07</td>
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Data are mean±SD.

*P<0.05 vs no cerebrovascular disease group; †P<0.01 vs before argatroban therapy.

![Figure 3. Change in NAA/Cre ratios in deep white matter in patients with chronic peripheral arterial occlusive disease 7 days after argatroban therapy. ○ indicates vascular dementia; ● indicates no vascular dementia.](image-url)

In Table 2, the changes in cerebral metabolism assessed by H-1 MRS are shown. The table compares the NAA/Cre ratio, Cho/Cre ratio, and MI/Cre ratio between the no cerebrovascular disease group and the advanced cerebrovascular disease group before and after argatroban therapy.

*Table 2. Changes in Cerebral Metabolism Assessed by H1-MRS*
Similarly, local thrombin generation at the site of injured cerebral vessel may also be precisely reflected as an increased TAT level. In addition, the effect of argatroban on the cerebral metabolism may be affected in part by the status of the destroyed blood-brain barrier. Thus, the improvement in cerebral metabolism caused by argatroban therapy may not have correlations with the reduction of intravascular thrombin generation or with the clinical improvement in PAOD. During argatroban therapy, even the suppression of a small amount of extravascular thrombin in the central nervous system may effectively improve cerebral metabolism so that argatroban easily penetrates the destroyed blood-brain barrier.

In conclusion, our results suggest that increased thrombin generation may have some pathophysiological roles in developing vascular dementia and its chronic predisposing conditions (multiple lacunar infarction and advanced PVH). Thrombin inhibition may break this vicious cycle and lead to clinical improvement.

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