White Matter Damage Is Associated With Matrix Metalloproteinases in Vascular Dementia

Gary A. Rosenberg, MD; Niky Sullivan, MSc; Margaret M. Esiri, DM, FRCPath

Background and Purpose—Vascular disease causes multi-infarct dementia (MID) or Binswanger’s disease (BD), the latter of which is a progressive form of vascular dementia (VaD) associated pathologically with fibrinoid and hyaline changes in brain arterioles with injury to the white matter. Clinically, BD patients have long-standing hypertension with disturbances of gait and intellect. Because matrix metalloproteinases (MMPs) are important in cerebral infarction, we hypothesized that disturbances in the MMPs may be involved in VaD.

Methods—Brain tissues from 5 patients with VaD of the BD or multi-infarct type (MID) were immunostained with antibodies to glial fibrillary acidic protein (GFAP), a microglial/macrophage cell marker (PG-M1), gelatinase A (MMP-2), stromelysin-1 (MMP-3), and gelatinase B (MMP-9). Control tissues were from 8 elderly patients: 4 with strokes without dementia and 4 without neurological diseases.

Results—PG-M1+ cells appeared around infarcts in patients with strokes without dementia and in patients with VaD. In 2 of the 3 BD patients, PG-M1 cells were prominent near damaged arterioles and scattered diffusely in white matter. MMP-2 was seen normally in perivascular macrophages and in astrocytic processes near blood vessels and was present in patients with strokes in reactive astrocytes. MMP-9 was rarely seen. MMP-3 was seen in PG-M1+ microglial/macrophage cells around the acute infarctions. In BD, MMP-3 persisted in tissue macrophages and disappeared in long-standing white matter gliosis.

Conclusions—These observations suggest that MMPs may participate in the damage to the white matter associated with VaD. Microglia/macrophage-induced damage, which is amenable to treatment, may be a factor in the progressive forms of VaD. (Stroke. 2001;32:1162-1168.)

Key Words: Binswanger’s disease ■ gelatinases ■ macrophages ■ metalloproteinases ■ microglia ■ stromelysin-1 ■ vascular dementia

Vascular disease causes dementia, and in the very old, it is as common as Alzheimer’s disease. Multiple strokes with loss of intellect occurring close to the time of the infarct are referred to as multi-infarct dementia (MID).1–3 Binswanger’s disease (BD) is a gradual deterioration with or without small strokes, often in association with hypertension.4,5 Pathologically, BD involves arteriolosclerosis with demyelination around the affected subcortical blood vessels and small subcortical infarcts.6 Brains of patients with BD have activated microglial cells with clusters of macrophages seen in areas with rarefied white matter.7 There is a buildup of extracellular matrix macromolecules that suggests an abnormality in matrix metabolism, which is normally regulated by the matrix metalloproteinases (MMPs) and the plasminogen activators.

MMPs are a gene family of >20 extracellular matrix–degrading neutral proteases.8 MMPs are secreted by astrocytes, endothelial cells, microglia, and neurons. Cerebral ischemia causes an elevation of gelatinase B (MMP-9) from 24 to 48 hours after the stroke, whereas gelatinase A (MMP-2) rises after 7 days, during the phase of wound healing and cyst formation.9 Gelatinases attack the basal lamina macromolecules, causing the proteolytic disruption of the blood-brain barrier.10 Another member of the MMP gene family, stromelysin-1 (MMP-3), has been observed in macrophages in multiple sclerosis and in neurons in Alzheimer’s disease.11,12 MMP-3 is highly disruptive to the extracellular matrix and has been associated with arthritis and the involution of breast tissue.9 Neutral proteases, including the MMPs, have been implicated in the breakdown of myelin.13 Recently, pathological studies of human autopsy material have demonstrated MMPs in acute stroke.14 Therefore, we hypothesized that excessive MMP activity, possibly related to reactive astrocytes or microglia/macrophages, would be present in brain tissues from patients with BD and would be related to perivascular demyelination. Brain tissues from patients with...
vascular dementia (VaD) were stained with antibodies to glial fibrillary acidic protein (GFAP), microglia/macrophages (PG-M1), MMP-2, MMP-3, and MMP-9. The intensity of the staining was graded, and regions with MMPs were correlated with vascular changes and white matter damage.

**Subjects and Methods**

Patients were selected from a series of patients with VaD that had other causes of dementia eliminated pathologically.6 Six patients with evidence of white matter disease and microinfiltrates were selected from 24 of those with dementia. Four patients were selected from 19 patients with cerebral infarcts but without dementia, and 4 were selected from 18 control patients without dementia. The age and sex of those in the 3 groups were comparable. Tissues from multiple regions had been obtained at the time of autopsy, and sections were available that had been previously stained with hematoxylin and eosin, Klüver-Barrera, Congo red, and various silver stains. Signed consent of a close relative for use of tissue for research was obtained for all material.

Clinical histories were reviewed, and the patients with dementia were separated into MID and BD. Those with infarctions due to large-vessel atheroma and sources of emboli, such as atrial fibrillation, were categorized as MID, and those with progressive dementia not necessarily related to infarctions, gait problems, and hypertensive arteriopathy or other causes of small-vessel disease were categorized as BD. Of the patients selected for the present study, 2 had histories and pathological findings compatible with MID, and 3 had histories and pathological findings compatible with BD.

Serial sections (10 μm) were obtained from the original blocks and immunostained with antibodies: GFAP (1:1000 for 1 hour at room temperature, Dako), PG-M1 (1:100 for 1 hour at room temperature, Dako), and monoclonal anti-human MMP-2 antibody (MAB902; 1:25 for 24 hours at 4°C, R&D Systems); a sheep anti-human MMP-3 (1:1000 for 24 hours at 4°C) that had worked well in paraffin-embedded tissues15 was a gift from H. Nagase (Imperial College, London, UK), MMP-9 (1:100 for 1 hour at room temperature) was also well characterized and was a gift from A. Gearing (British Biotechnology, Oxon, UK). Sections were immunostained after blocking endogenous peroxidase with 3% hydrogen peroxide (30 minutes) and microwave pretreatment in citrate buffer, pH 6.0. Application of antibodies was preceded by exposure of sections to 20% FCS for 20 minutes. Primary antibodies were followed by 2 washes in PBS, by a second-layer antibody at 1:200 for 30 minutes at room temperature, by Vector ABC (Vector Laboratories) according to kit instructions, and then by the metal-enhanced diaminobenzidine reaction. Sections were then weakly counterstained with hematoxylin, mounted, and coverslipped.

A grading system was established to grade the extent of staining with each antibody, ranging from no stain (a score of 0) to extensive staining (a score of 4). Whether the staining was focal or diffuse, the region of the focal staining was also noted. Sections stained previously were used to correlate MMPs with myelin loss, small-vessel arteriopathy, amyloid deposition, and silver impregnation.

**Results**

The clinical features of the patients are shown in Table 1, and semiquantitative grading scores are shown in Table 2. In the demented group, 2 patients had large-vessel disease and multiple strokes, consistent with the diagnosis of MID. The other 3 demented patients had small-vessel disease and a progressive course, which began with gait disturbance in 1 patient but eventually involved the gait in all patients. The 4 patients that experienced cerebrovascular accidents (CVAs) all had ≥1 stroke but did not have dementia. Small-vessel pathology was absent, but 3 showed large-vessel atheromas. The tissue was collected from 1972 to 1994, with the majority obtained in the early 1980s, when brain neuroimaging had not been routinely performed.

**Control Subjects Without Neurological Diseases**

Control brains showed small numbers of GFAP-positive (GFAP+) reactive astrocytes scattered throughout the gray and white matter. In the gray matter, GFAP was found around blood vessels and in the subpial regions. Occasionally, a clump of GFAP+ cells was seen in the cortex, which corresponded with silver-stained Alzheimer-type plaques. The microglial marker, PG-M1, showed rounded cells at-

| TABLE 1. Clinical and Pathological Findings in Patients With and Without Dementia and in Control Subjects |
|---|---|---|---|---|---|---|
| Initials | Age, y | Sex | Clinical Diagnosis | Small Vessel | Large Vessel | Gait Problem | Other Diseases |
| T.R. | 83 | M | MID | No | Yes | No | IHD, AF, PD |
| M.H. | 76 | F | MID | No | No | No | CHF, AF, AMT |
| S.B. | 62 | M | BD | Yes | No | No | HypoThy, DVT, PA |
| L.C. | 74 | M | BD | Yes | No | Yes | HYP |
| M.T. | 66 | F | BD | Yes | No | No | HYP, NPH |
| E.E. | 81 | F | CVA | No | Yes | No | AF |
| J.P. | 78 | M | CVA | No | Yes | No | MI |
| V.R. | 83 | F | CVA | No | Yes | No | Asthma |
| A.R. | 86 | M | CVA | No | No | No | None |
| C.L. | 92 | M | Control | No | No | No | Prostate CA |
| J.G. | 72 | M | Control | No | No | No | ETOH |
| M.Wa. | 76 | F | Control | No | No | No | Ovarian CA |
| M.Wi. | 84 | F | Control | No | No | No | Unknown |

IHD indicates ischemic heart disease; AF, atrial fibrillation; PD, Parkinson’s disease; CHF, congestive heart failure; AMT, acute terminal meningitis; HypoThy, hypothyroidism; DVT, deep-vein thrombosis; PA, pernicious anemia; HYP, hypertension; NPH, normal-pressure hydrocephalus; MI, myocardial infarction; CA, cancer; and ETOH, alcoholism.
tached to the blood vessels, along with the resting parenchymal microglia with short thin processes.

A small focus of white matter damage was seen around a subcortical blood vessel in 1 of the normal patients (Figure 1A). Clusters of PG-M1–positive (PG-M1+) cells were seen in these regions (Figure 1B). Immunoreactivity for MMP-2 and MMP-3 was present in a few of these cells (Figure 1C and 1D), but MMP-9 was absent. MMP-2 immunostaining was seen in subpial astrocytes (Figure 1E), in subependymal (Figure 1F) regions, and next to cerebral blood vessels. Vessel-associated cells had MMP-2 reactivity and resembled those described as perivascular macrophages or pericytes. Perivascular cells also stained for MMP-3, but it was difficult to determine if they were the same cells. MMP-9 staining was seen only in occasional neutrophils within the lumen of the blood vessels in control brains.

CVA Without Dementia

Occasional GFAP+ cells were found in the gray and white matter of patients with CVA without dementia as in control subjects, but around the regions of infarction, a marked build-up of GFAP+ cells was observed. Large-bodied astrocytes with thick processes that resembled gemistocytes were located at the edge of the infarcted tissue (Figure 2A). These cells were intensely MMP-2 positive (Figure 2B). In cystic regions in which blood vessels were either preserved or regrowing, these reactive astrocytes had processes attached to the vessels.

Microglial/macrophage cells that stained with PG-M1 were prominent in the region of the infarct, where they were located both in the cystic necrotic regions and further away in the parenchyma surrounding those regions. White matter showed the highest number of reactive microglia. Occasional bands of demyelination radiated from the ventricle, leaving adjacent white matter intact (Figure 3A). Round-bodied macrophages were prominent in these demyelinated regions, sparing the contiguous gray matter (Figure 3B). Cells staining

<table>
<thead>
<tr>
<th>Initials</th>
<th>Diagnosis</th>
<th>GFAP</th>
<th>PG-M1</th>
<th>MMP-2</th>
<th>MMP-3</th>
<th>MMP-9</th>
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<td>MID</td>
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<td>3</td>
<td>2</td>
<td>3</td>
<td>1-F</td>
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<tr>
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<td>MID</td>
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<td>3</td>
<td>2</td>
<td>3</td>
<td>3-Men</td>
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<tr>
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<td>1</td>
<td>0</td>
<td>3</td>
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<tr>
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<td>3</td>
<td>1</td>
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</tr>
<tr>
<td>M.T.</td>
<td>BD</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>E.E.</td>
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<td>0</td>
<td>2-F</td>
<td>1</td>
<td>2-F</td>
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<tr>
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<td>CVA</td>
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<td>2-F</td>
<td>3-F</td>
<td>1-F</td>
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<tr>
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<td>3-F</td>
<td>3-F</td>
<td>2-F</td>
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</tr>
<tr>
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<td>CVA</td>
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<td>3-F</td>
<td>0</td>
<td>2-F</td>
<td>0</td>
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<tr>
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<td>1</td>
<td>1-Pial</td>
<td>1-Peri</td>
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<tr>
<td>J.G.</td>
<td>Control</td>
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<td>0</td>
<td>1-Pial</td>
<td>1-Peri</td>
<td>0</td>
</tr>
<tr>
<td>M.Wa.</td>
<td>Control</td>
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<td>1</td>
<td>1-Pial</td>
<td>1-Peri</td>
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<tr>
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<td>Control</td>
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<td>1</td>
<td>1-Pial</td>
<td>1-Peri</td>
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F indicates focal; Peri, perivascular; and Men, meningitis. Grading system is as follows: 0, none; 1, minimal; 2, scattered; 3, marked; and 4, confluent.

Figure 1. Small demyelinated region in a control brain. A, Myelin stain showing blood vessel with enlarged perivascular space and inflammatory cells near the region of demyelination (original magnification ×100). B, Same region as in panel A (original magnification ×100). Insert is higher power of demyelinated region and indicates the presence of PG-M1+ cells (original magnification ×400). C, MMP-2+ cells (arrows) in a low-power field (original magnification ×100) with insert showing several PG-M1+ cells (original magnification ×400). D, Same area as in panel A showing MMP-3 staining in cells in the same area (original magnification ×100). Insert is higher power (original magnification ×400). E, Subpial zone with MMP-2 reactivity in cells near penetrating blood vessel (arrow) (original magnification ×400). F, Ependymal cells with MMP-2+ cells (arrows) beneath them and fine processes extending through them into the ventricle (original magnification ×400).
for PG-M1 were seen in regions with cells staining for MMP-3 (Figure 3C). The MMP-3–containing cells were large with prominent nuclei, suggesting that they were tissue macrophages (Figure 3D). Outside the infarcted and immediately surrounding regions, MMP-2 had the appearance seen in the control brains. Many microglial/macrophage cells showed intense staining for MMP-3, and a diffuse brown color was seen in the tissues, which may have been an artifact. In the cystic regions, blood vessel endothelium showed MMP-3 immunoreactivity. MMP-9 staining was absent except for around a rare blood vessel.

**MID and BD**

Around the infarcted tissue, the MID group showed a pattern of MMPs similar to that seen in the CVA group. In the noninfarcted tissues, the gray matter appeared the same as in the control subjects. However, the white matter showed more extensive involvement by GFAP-containing reactive astrocytes. The closer the regions were to the infarcts, the greater the GFAP response. In the region of the infarct, the MID patients showed many PG-M1+ macrophage-like cells. MMP-3–positive (MMP-3+) cells were seen in the vicinity of those reacting to PG-M1. However, these cells were also seen in the white matter regions close to the stroke region. MMP-2–immunoreactive product was restricted to the regions around the cerebral blood vessels, particularly in the large astrocytes in the vicinity of the infarcts but also in a few macrophage-like cells. In the meningeal regions overlying the infarcts, the subpial showed a more intense response to MMP-2, with many subpial astrocytes and their foot processes showing MMP-2 immunostaining.

In the BD patients, prominent staining for MMP-3 in tissue macrophages was seen in a diffuse pattern in many white matter regions. The accumulation of MMP-3+ cells tended to cluster around thickened blood vessels (Figure 4A). Many of the cells close to the blood vessels had the appearance of macrophages (Figure 4B and 4D). However, in the BD brains, clusters of MMP-3+ cells were also observed in white matter distant from the involved blood vessels (Figure 4C).

All of the BD patients showed diffuse GFAP reactivity in the white matter. This extensive gliosis was seen in regions close to small infarcts and also in those far away. Two of the 3 patients had PG-M1+ cells in many areas of white matter, with the microglial/macrophage response accentuated around infarcted regions. However, the third BD patient, who had the longest course (>15 years), had extensive demyelination with sparing of the U fibers (Figure 5A), and the GFAP+ cells were small with thin processes (Figure 5B). Staining for PG-M1 was seen only in scattered cells (Figure 5C), and MMP-2 (Figure 5D) and MMP-3 (Figure 5E) were restricted to the perivascular regions. Control sections from which primary antibody was omitted were entirely negative.

**Discussion**

Brain tissue from patients with VaD showed a microglial/macrophage neuroinflammatory response similar to that seen in the MID group.
in patients with strokes and no dementia, but there was a more diffuse pattern in the white matter. Microglial/macrophage cells expressed high levels of MMP-3, which was particularly prominent around arterioles with fibrohyaline degeneration in the white matter and scattered in the parenchyma. MMP-2 was seen in these regions of chronic inflammation in reactive astrocytes and, to a lesser extent, in microglia/macrophages. Very little MMP-9 was seen. The findings in the patients with clinical features of MID were of the same sort but were more localized to regions around the infarctions. Our results suggest that ongoing white matter injury, related particularly to MMP-3–producing microglia/macrophages, occurs in patients with BD.

White matter damage was seen to evolve around fibrotic hypertrophied blood vessels with cells that stained for PG-M1, suggesting that they were microglia/macrophages. Microglia form several potentially toxic substances that could have damaged the white matter, including free radicals and proteases. In the vicinity of those cells were MMP-3–containing cells that resembled tissue macrophages. One of the control subjects had 1 site of loss of myelin stain with PG-M1+ and MMP-3+ cells. In the patients without dementia but with cerebral infarction, the regions adjacent to the infarction also contained these cells. However, the demented patients had PG-M1+ cells in multiple white matter regions. Those with MID had these cells more prominently around the infarct, whereas the BD patients had a more widespread distribution.

The most prominent staining in the pathological material was seen with the antibody to MMP-3. One patient with acute

Figure 4. MMP-3–containing cells in BD subject. A, Arteriosclerotic vessel with fibrohyaline changes. MMP-3+ cells are seen surrounding the vessel and in the adjacent parenchyma (arrows) (original magnification ×100). B, Large tissue macrophages with MMP-3 in severely affected white matter region (original magnification ×200). C, Area of demyelination distant from a blood vessel with intense MMP-3+ cells (original magnification ×400). D, Area closer to a blood vessel showing many MMP-3+ tissue macrophages (original magnification ×400).

Figure 5. Chronic changes in patient with long-standing BD (≈15-year duration of the illness). A, Whole-mount myelin-stained section showing extensive demyelination with sparing of the U fibers. B, GFAP+ astrocytes (arrows) in an intensely gliotic area. The astrocyte cell body is small, and the fibers are thin (compare with Figure 2) (original magnification ×400). C, Sparse PG-M1 in rare perivascular macrophages and scattered microglia in the vicinity of the blood vessel in the gliotic white matter (original magnification ×100). D, Rare MMP-2+ perivascular cells (arrow) (original magnification ×100). E, MMP-3 staining in only 1 perivascular cell (arrow) (original magnification ×100).
multiple sclerosis had MMP-3 immunoreactivity in macrophages.\textsuperscript{11} MMP-3+ cells were found in regions of damaged white matter, where PG-M1+ cells were also seen. Although there is an increase in PG-M1+ and MMP-3+ cells, which is consistent with a microglial/macrophage response, the role of MMP-3 remains to be determined. Furthermore, the origin of the PG-M1+ cells could not be determined, and they could be derived from the microglial cells or from peripheral macrophages.\textsuperscript{16} In experimental ischemia in the rat, dual-label immunohistochemistry showed MMP-3+ microglia/macrophages and neurons.\textsuperscript{17}

In the earlier reports on MMPs in stroke, MMP-9 was seen in the acute animal and human studies. The present study failed to show evidence of MMP-9 in the chronic patterns of injury observed in VaD. Instead, we observed increased MMP-3, which suggests that this may be more important in the long-term changes. A complex interaction of the MMPs has been observed in experimental animals with stroke.\textsuperscript{17} In the first 24 to 48 hours after the infarction, there is an increase in MMP-9 and MMP-3. The MMP-3 is seen in the microglia/macrophages and is thought to be activated by plasmin. Once activated, the MMP-3 activates the latent form of MMP-9. It is possible that MMP-3 in the chronic stages is acting alone in damaging the extracellular matrix, but further studies will be needed to elucidate the exact role of MMP-3 in the brain.

The present study is preliminary, contains a relatively small number of patients, and uses retrospective clinical information. However, consistent patterns of MMP reaction were observed, suggesting that these enzymes may have a role in BD. Material from a larger number of patients prospectively studied will need to be examined to confirm these preliminary observations. If they are confirmed, they will place some forms of VaD in the category of an inflammatory condition related to a microglial/macrophage response.

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References


Editorial Comment

Matrix Metalloproteinases and Diffuse White Matter Injury

Matrix metalloproteinases (MMPs) are secreted proteolytic enzymes that mediate extracellular matrix molecule turnover in a wide variety of tissues. The extracellular matrix alterations mediated by MMPs are critical for normal development and tissue homeostasis and are central to a range of pathological processes such as atherosclerosis, inflammation, angiogenesis, and neoplasia. In the central nervous system (CNS), they have potentially critical roles in the pathogenesis of major diseases, including multiple sclerosis (MS), Alzheimer’s disease, and gliomas.\textsuperscript{1,2} In this immunohistochemical study of brain samples from patients with multi-infarct dementia, Binswanger’s disease (BD), and other conditions, the authors demonstrate enhanced glial fibrillary acidic protein–positive astrocytes and microglia around affected arterioles and persistently elevated detection of MMP-3 on macrophages in BD but not in stroke or control brains. These data implicate MMP activation in the pathogenesis of white matter injury in BD and related disorders.

The study is somewhat limited both by the relatively small number of examples of each disorder and the necessarily...
restricted scope of the analysis; ie, 3 of the more than 20 MMPs that have been identified to date were evaluated. The authors appropriately acknowledge that their findings are preliminary. Moreover, how the increased MMP expression relates specifically to axonal and myelin pathology could not be precisely determined. Nevertheless, the authors’ conclusions are justified by the data presented and are consistent with experimental stroke studies3,4 and studies of CNS inflammatory conditions in which MMP-mediated remodeling of the extracellular matrix is suggested.5,6

There are several important clinical and pathogenetic implications of these findings. First, this study adds BD and multi-infarct dementias to the growing list of CNS diseases that may be amenable to specific pharmacological targeting of MMP activities.7 Other therapeutic modalities for stroke, such as mild hypothermia, may offer cerebroprotection to patients with diffuse white matter injury, in part through reduction of inflammatory cascades that include MMP activation.8 On the other hand, however, treatments appropriate for acute ischemia and primary inflammatory processes may be less feasible for these chronic disorders.

In addition, the demonstration of MMP activation in these diseases provides new insight into their poorly understood pathophysiology. In contrast to the MMP activation of focal lesions, such as occurs in infarcts and MS plaques, the present findings suggest more widespread activation of microglia/macrophage-derived MMPs than previously appreciated. Furthermore, this activation appears to be a consequence of the arteriolar pathology of BD rather than, as in MS, of primary, overt inflammatory cell infiltration. Diffuse MMP activation and gliosis could result in subtle white matter injury in areas distant from overt lesions through molecular mechanisms similar to those that occur in association with larger lesions and that lead to cavitation.9 Widespread, diffuse MMP activation and altered extracellular matrix turnover in BD may, therefore, contribute to white matter dysfunction that is manifested clinically by dementia in the absence of either extensive cerebral cortical pathology (as in Alzheimer’s disease) or larger destructive lesions (as in multi-infarct dementia, trauma, and MS).

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