Measurement of Gelatinase B (MMP-9) in the Cerebrospinal Fluid of Patients with Vascular Dementia and Alzheimer Disease

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Background and Purpose—Vascular causes of dementia are increasing in importance because of the aging of the population. Biological markers to distinguish patients with vascular dementia (VaD) from Alzheimer disease (AD) would be very useful. Because cerebrovascular disease increases expression of brain matrix metalloproteinases (MMPs) and tissue inhibitors to metalloproteinases (TIMPs), we hypothesized that MMPs would be elevated in the cerebrospinal fluid (CSF) of patients with VaD, but not in patients with AD.

Methods—Fifteen patients with VaD were identified, including dementia caused by multiple infarcts and progressive dementia caused by disease of the small cerebral blood vessels. Patients were followed-up for 4 to 10 years to confirm the diagnosis. Thirty patients with AD were also studied. Patients had CSF collected at their initial evaluation. Gelatinase A (MMP-2) and gelatinase B (MMP-9) were quantified by gelatin-substrate zymography, and TIMPs were measured by reverse zymography. Control CSF was obtained from neurologically normal subjects.

Results—MMP-9 levels were significantly elevated in the CSF of VaD patients compared either to those with AD (P < 0.0001) or to controls. MMP-2, TIMP-1, and TIMP-2 were similar in patient groups and controls.

Conclusions—Patients with multiinfarct and small vessel VaD have elevated levels of MMP-9 in the CSF compared with AD and controls. Although CSF MMP-9 increases in other neurological conditions and is not specific for VaD, it could provide an additional biological marker for the separation of patients with VaD and AD. (Stroke. 2004;35:000-000.)

Key Words: matrix metalloproteinase ■ dementia
(UNMH), the New Mexico Regional Veterans Administration Medical Center (NMVAMC), and the Oregon Health Sciences University (OHSU). Patients from UNMH and NMVAMC with dementia caused by multiple infarctions according to the criteria of Chui et al.,7 or withBinswanger disease according to the criteria of Caplan and Schoene,8 were recruited into the study. Longitudinal follow-up over 4 to 10 years was performed during subsequent hospital admissions, clinic visits, or by record review to increase diagnostic certainty.

Cerebrospinal fluid was collected at the time of initial evaluations. All CSF specimens were aliquoted at the time of collection and frozen at −80 °C for subsequent analysis; none was thawed and refrozen. All samples were analyzed at the same time, allowing comparison of specimens collected at different times. Thirty specimens from patients with AD were obtained from the OHSU Aging and Alzheimer’s Disease Center. Eight control CSF samples came from age-matched patients without history of neurological disorders who were undergoing spinal anesthesia at UNMH. All patients consented to have the CSF samples studied.

Gelatin-Substrate Zymography and Reverse Zymography

MMP-2 and MMP-9 were measured in CSF by gelatin-substrate zymography as described earlier.9 In brief, CSF was placed on 10% sodium-dodecyl sulfate (SDS) polyacrylamide minigels copolymerized with gelatin. Protein standards (GIBCO) and HT1080 fibrosarcoma media, which contain MMP-2 and MMP-9, were used in every gel to determine the molecular weight of detected gelatinases. To verify that bands were from MMPs, additional gels were incubated with EDTA without calcium. The assay’s accuracy was determined by Western immunoblots. Neither of the TIMP activ-

Tissue inhibitors to metalloproteinases were detected in CSF samples by reverse zymography. Polyacrylamide minigels (15%) were prepared with gelatin and purified MMP. Samples were mixed 1:1 with nonreducing SDS buffer (New England Biolabs). Prestained molecular weight markers (Amersham Life Science) and HT1080 fibrosarcoma media were used in every gel.

Gels were digitized using a scanner (HP scan II; Hewlet-Packard) and a computer-based imaging system was used to measure relative lysis areas (NIH Image on a Macintosh Power PC).

Statistical Analysis

Statistical analysis was performed with Prism 3.0 statistical software (GraphPad Inc). Student t tests and Mann–Whitney U tests were used for statistical comparisons. Linear regression was used to correlate measurements taken over time. Significance was set at \( P<0.05 \). All values are given as mean±standard error of the mean (SEM).

Results

Clinical Data

Of the 15 patients with VaD, 8 patients had a slowly progressive course associated with hypertension, focal find-

Figure 1. Quantitative zymography data from UNMH patients. A, Levels of MMP-2 for the VaD patients compared with controls; no statistical differences were found. B, MMP-9 in the VaD patients compared with the controls; MMP-9 was significantly higher than controls as shown by the asterisk (Student \( t \) test; \( P<0.003 \)).
Likewise, there was no group difference with regard to TIMP activity. Comparison of the VaD data from UNMH with the AD data from OHSU was accomplished through normalizing each sample by the controls’ mean values, because the same control samples were used separately with each patient set. Normalized data showed that MMP-9 levels from the VaD group were significantly higher than values from AD patients ($P<0.0047$) (Figure 3).

**Discussion**

VaD caused an increase in the levels of MMP-9 in the CSF. Patients with AD did not show a similar increase and had values in the control range. Diagnostic criteria for VaD are controversial because of the group of patients with a slowly progressive course and damage to the myelin secondary to the vascular disease. Because VaD is a heterogeneous disorder and can follow a variable course, diagnostic criteria for the large vessel, multi-infarct, and small vessel forms of the illness were used. Recently, a combination of several previous diagnostic criteria has been proposed, and these new criteria fit our patient groups. Because autopsies were not performed, the diagnosis was confirmed by long-term follow up. In this series, approximately half of VaD patients had a progressive course consistent with Binswanger disease, whereas the others had a course more consistent with multiple infarcts. This breakdown of patients is similar to that found in an autopsy series. Elevation of MMP-9 in the CSF is a nonspecific finding reported in a number of neuroinflammatory conditions, including multiple sclerosis, AIDS dementia, and viral infections. The present study is the first description, to our knowledge, of elevated MMP-9 levels in patients with VaD. There are several sources for MMP-9 in the CSF: extravasation from the blood, release by infiltrating leukocytes, and endogenous production by brain cells. A recent report described elevated levels of MMP-9 in the plasma in patients with AD. However, in our series and in 1 other published report, levels of MMP-9 were not found to be elevated in the CSF in AD patients. In patients with VaD, the CSF cell count was normal, but protein was elevated, suggesting an abnormality of the blood–brain barrier. Accordingly, some of the MMP-9 in the CSF may come from the blood. The lack of a change in MMP-2 levels argues against this account, but confirmation must await further studies that index CSF levels to those in the blood.

In an autopsy study of patients with VaD, stromelysin-1 (MMP-3) and MMP-2 were the major MMPs detected. One possible explanation for finding an acute marker of inflammation (ie, MMP-9) in the CSF of patients with a chronic disease is that they were studied during a clinically active phase. Longitudinal analysis in several patients showed a gradual decrease in MMP-9, consistent with the autopsy findings.

Although the number of patients in the present study was small, the differences between the VaD and AD patients were robust. The results suggest that CSF MMP measurements, although not diagnostic, might be combined with factors such as clinical course, psychometric profile, and imaging results to improve the early distinction between VaD and AD, potentially improving patient selection in future clinical trials.

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**References**


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