Expression of matrix metalloproteinases and their tissue inhibitors in the serum and cerebrospinal fluid of patients with meningitis

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Abstract

Meningitis is associated with an imbalance between matrix metalloproteinases (MMPs) and endogenous tissue inhibitors of MMP (TIMPs). Serum and CSF were collected prospectively from all patients with meningitis between January 2008 and December 2008 to measure the concentrations of MMP/TIMP in those patients who underwent a lumbar puncture for a presumptive diagnosis of meningitis. A total of 199 patients were enrolled into the study. The concentrations of CSF MMP-9 and TIMP-1 were significantly higher in the meningitis group compared with the control group (p 0.032 and p <0.001, respectively). However, the CSF TIMP-4 levels were significantly lower in the meningitis groups compared with the control groups (p <0.001). Patients with bacterial meningitis had higher CSF MMP-9 and TIMP-1 levels than those who had aseptic meningitis and controls. Patients with various infectious meningitis etiologies tended to have higher CSF MMP-9 expression by gelatin zymography when compared with the controls. In conclusion, MMP/TIMP system dysregulation was found in patients with meningitis, and CSF MMP and TIMP might act as novel indicators in patients with meningitis.

Keywords: Matrix metalloproteinase, meningitis, tissue inhibitors of matrix metalloproteinase, zymography

Original Submission: 1 April 2010; Revised Submission: 6 August 2010; Accepted: 21 September 2010
Editor: M. Drancourt
Article published online: 14 October 2010
Clin Microbiol Infect 2011; 17: 780–784
10.1111/j.1469-0691.2010.03393.x

Meningitis is the most common serious infection of the central nervous system (CNS). The case mortality rate is relatively high and survivors may suffer from long-term, severe neurological sequelae [1–4]. In bacterial meningitis, acute breakdown of the blood–brain barrier (BBB) and accumulation of blood-derived leukocytes in the cerebrospinal fluid (CSF) lead to brain edema, cerebral vasculitis and ultimately neuronal injury [5]. One of the prototypical destructive events in the human brain, initiated by the release of inflammatory cytokines and ending with tissue destruction, is the production of matrix metalloproteinases (MMPs) [5].

The MMPs constitute a family of zinc-binding endopeptidases characterized by their ability to degrade various extracellular matrices [6]. The activity of MMP is highly regulated both at the level of gene expression and by conversion of latent pro-MMP to active enzymes [7]. Infectious meningitis is associated with an imbalance between MMPs and endogenous tissue inhibitors of MMP (TIMPs) [8,9]. In the extracellular milieu, the activity of these enzymes is controlled by four natural TIMPs. Changes in the fine balance between MMPs and their tissue inhibitors drives extracellular matrix turnover and may be associated with inflammation and neurotoxicity [10]. The manner in which the matrix-degrading enzymes and their TIMP inhibitors are regulated in the microenvironment of the brain of humans with meningitis is not well understood. This study was designed to obtain clues concerning the potential roles of MMPs/TIMPs in patients with meningitis.

Eligible subjects were enrolled into a prospective clinical cohort of patients with suspected meningitis in the Department of Infectious Diseases at Kaohsiung Veterans General Hospital between January 2008 and December 2008. The diagnosis of bacterial meningitis was based on detection of the pathogen in the CSF by gram staining, bacterial culture or antigen testing, and the presence of CSF pleocytosis [11]. Tuberculous (TB) meningitis was established on the basis of the presence of symptoms and/or signs suggestive of meningitis plus one of the following criteria: (i) positive culture of Mycobacterium tuberculosis, positive smear for acid-fast bacilli (AFB), or positive polymerase chain reaction (PCR) from the CSF; (ii) positive culture of Mycobacterium tuberculosis, positive smear for AFB, or positive PCR from other body fluids or organs; and (iii) negative CSF culture for virus, bacteria and fungi, plus clinical
response to anti-tuberculosis therapy [12]. Cryptococcal meningitis was diagnosed based on a positive CSF India ink staining or a positive culture for the organism or a positive cryptococcal antigen latex agglutination test [13]. Aseptic meningitis was defined as patients with a positive CSF viral culture or pleocytosis in the CSF with negative CSF bacteriological studies, or pleocytosis in the CSF with viral isolation from other sources such as a throat or a rectal swab [14]. A human immunodeficiency virus (HIV)-infected patient with neurosyphilis was defined as having a positive HIV Western blot test and a reactive serum rapid plasma reagin (RPR), as well as a CSF white blood cell count ≥20 cells/μl or a reactive CSF Venereal Disease Research Laboratory (VDRL) [15].

Lumbar punctures were performed if patients had neurological or ophthalmological symptoms/signs, change in consciousness, ataxia, fever and/or meningeal sign. The CSF control group (n = 111) consisted of patients with headache or disturbed consciousness who underwent a lumbar puncture for exclusion of tumour, subarachnoidal haemorrhage, inflammatory disease or meningitis. The CSF samples were centrifuged and the supernatants were frozen at −80°C until assayed. The study protocol, including informed consent, was approved by the Institutional Review Board of the Kaohsiung Veterans General Hospital.

Concentrations of MMP-2, MMP-9, TIMP-1, TIMP-2 and TIMP-4 in serum and CSF were determined using ELISA Kits (R & D Systems, Minneapolis, MN, USA) according to the manufacturer’s instructions [9]. Activity of MMPs was analysed by a modified sodium dodecyl sulfate–polyacrylamide gel electrophoresis [9].

All of the data were expressed as mean ± standard deviation. Concentrations of MMP-2, MMP-9, TIMP-1, TIMP-2 and TIMP-4 in CSF and serum in patients with meningitis and controls were compared by Student t-test or Mann–Whitney U-test. The mean concentrations of MMP-2, MMP-9, TIMP-1, TIMP-2 and TIMP-4 in CSF for the different meningitis aetiologies were compared by one-way analysis of variance followed by the Scheffe’s multiple range test. A Pearson correlation test was used to compare the levels of CSF parameters and concentrations of MMPs/TIMPs. The results were presented as the mean ± SD. A p value <0.05 was considered statistically significant.

Between January 2008 and December 2008, a total of 199 patients with suspected meningitis were enrolled into this prospective study, and the concentrations of MMP/TIMP in the cerebrospinal fluid (CSF) and serum were measured. The mean ages (y/o) of the meningitis and control groups were not different (52.2 ± 20.2 (range 16–87) and 63.3 ± 19.6 (range 20–96), respectively). Most of them were men (67.4% vs. 67%). Twenty-five patients had bacterial meningitis, 11 had TB meningitis, 11 had cryptococcal meningitis, 35 had aseptic meningitis, and six patients had HIV and neurosyphilis. For those 25 patients with bacterial meningitis, 20 patients had a definite aetiological diagnosis, and in the other five the diagnosis was based on the presence of bacteria on Gram’s stain of the CSF. Causal pathogens included Klebsiella pneumoniae in seven, Streptococcus pneumoniae in three, Listeria monocytogenes in two, and one each of Streptococcus salivarius, Streptococcus pyogenes, Streptococcus agalactiae, Enterococcus, Pseudomonas aeruginosa, E. coli, Acinetobacter baumannii and Staphylococcus aureus. The control group consisted of 111 patients without meningitis, presenting with headache or disturbance of consciousness, and who underwent a diagnostic lumbar puncture. The diagnosis in the control group included septic shock in 52, metabolic encephalopathy in 32, Guillain–Barré syndrome and vasculitis in seven, stroke in five, infective endocarditis in four, malignancy in four and HIV infection in four. Three patients had herpes zoster infection.

The CSF concentrations of MMP-9 and TIMP-1 were significantly higher in the patients with meningitis compared with the controls. The CSF concentrations of TIMP-4 were significantly lower in the patients with meningitis compared with the controls (p <0.0001) (Table 1). Serum concentrations of MMPs and TIMPs were not significantly different between patients with meningitis and the controls. Further subgroup analysis found significant differences in the concentrations of MMP-9 and TIMP-1 between patients with bacterial meningitis and those who had aseptic meningitis and controls. Patients with bacterial meningitis tended to have higher CSF MMP-9 and TIMP-1 levels compared with other meningitis aetiologies and controls Table 1.

We examined the correlation of CSF white cell count with MMP-9 concentrations in patients with infectious meningitis. Levels of MMP-9 in the CSF of patients significantly correlated with the levels of CSF protein (r = 0.257, p 0.001), CSF white cell count (r = 0.358, p <0.001), CSF MMP-2 (r = 0.671, p <0.001), CSF TIMP-1 (r = 0.311, p <0.001) and CSF TIMP-2 (r = 0.256, p <0.001) (Table 2). The higher the white cell count level in the CSF of patients, the higher the MMP-9 concentrations in the CSF. Levels of CSF TIMP-4 were negatively associated with the concentrations of CSF protein (r = −0.167, p 0.041) and CSF TIMP-1 (r = −0.229, p 0.003). We also found a strong correlation between CSF protein and concentrations of CSF MMP-2 and 9 and TIMP-1, 2 and 4. The CSF white cell count was correlated with levels of CSF MMP-2 and 9, and TIMP-1 and 2, but not with TIMP-4 Table 2.

The CSF specimens from five of the patients and one of the controls were randomly selected for analysis by gelatin...
zymography. All CSF specimens from patients and controls demonstrated a band with a molecular mass of 72 kD (MMP-2). However, only CSF specimens from patients with infectious meningitis showed MMP-9 activity with a band with a molecular mass of 92 kD.

MMP-9 in the CSF has been proposed as a marker of meningitis, neurotropic viruses and neuroinflammation [8,9,16]. MMP-9 has the capacity to degrade endothelial basement membrane components, and is of strategic importance in the migratory processes into the CNS [17]. Elevated concentrations of MMP-9 have been reported to alter the blood-brain barrier, leading to brain barrier damage, neuroinflammatory processes and meningeal oedema [5,8,9].

The brain appears to be the source of the elevated MMP-9 in the CSF of patients with meningitis. This hypothesis is based on our findings that the serum concentrations of MMP-9 in patients with infectious meningitis did not differ from non-meningitis controls. There is evidence that MMP-9 is produced by infiltrating immune cells, ependimocytes, microglia [18,19] and other parenchymal or endothelial cells [20]. Animal models and clinical studies of bacterial meningitis also suggested degranulation of polymorphic leukocytes (PMNs) as the primary source of CSF MMP-9 [8,20,21].

Conflicting results have been reported with regard to the relationship between CSF pleocytosis and MMP-9 levels. A relationship between increased MMP-9 levels in CSF and the number of leukocytes in various neurological disorders was first reported by Gijbels and others [22]. In contrast, Paemen and others [16] studied inflammatory neurological diseases such as optical neuritis and multiple sclerosis and did not find a significant correlation of MMP-9 with CSF leukocyte counts. In this study, a highly significant correlation was found between CSF MMP-9 concentrations and CSF leukocyte counts and protein. It is likely that increased concentrations of MMP-9 in CSF were derived from the transmigrating leukocyte cells because of damage to the blood-brain barrier (BBB). These results suggest that increased MMP-9 levels are associated with BBB dysfunction and are dependent on CSF pleocytosis. A significant increase in CNS concentrations of TIMP-1 has been observed in infectious meningitis [8,9]. Increased expression of MMP-9 and TIMP-1 in CSF of bacterial meningitis, viral meningoencephalitis and eosinophilic meningitis has also been reported by several groups [8,9,20,23]. The finding that concentrations of TIMP-1 were ten to hundreds-fold higher than MMP-9 in patients with viral [23] and eosinophilic meningitis [9] and in our patients, suggests that MMP-9 is bound to TIMP-1 [24]. In a mouse brain experimental autoimmune encephalomyelitis (EAE) model, Pagenstecher et al. [25] showed that upregulation of MMP-9 was associated with marked enhancement of

### Table 1: Comparison of CSF MMP-2 and 9 and TIMP-1, 2 and 4 concentrations in patients with infectious meningitis and controls

<table>
<thead>
<tr>
<th>Condition</th>
<th>MMP-2 (ng/mL)</th>
<th>MMP-9 (ng/mL)</th>
<th>TIMP-1 (ng/mL)</th>
<th>TIMP-2 (ng/mL)</th>
<th>TIMP-4 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial meningitis (n=25)</td>
<td>102.3±45.0</td>
<td>297.6±275.5</td>
<td>342234.1±153127.8</td>
<td>71504.8±29369.8</td>
<td>1036.9±743.9</td>
</tr>
<tr>
<td>TB meningitis (n=11)</td>
<td>81.3±50.8</td>
<td>247.7±215.1</td>
<td>326475.2±137928.8</td>
<td>81940.6±22690.8</td>
<td>634.3±389.0</td>
</tr>
<tr>
<td>Cryptococcus meningitis (n=11)</td>
<td>73.3±39.0</td>
<td>217.2±15.5</td>
<td>253481.4±159193.7</td>
<td>6982.6±1818.2</td>
<td>4738.1±2491.9</td>
</tr>
<tr>
<td>Aseptic meningitis (n=35)</td>
<td>57.9±30.2</td>
<td>121.2±15.5</td>
<td>182839.7±124697.8</td>
<td>57682.5±17429.8</td>
<td>1327.9±717.3</td>
</tr>
<tr>
<td>HIV with neurosyphilis (n=6)</td>
<td>42.1±17.7</td>
<td>80.7±67.7</td>
<td>118944.1±99766.3</td>
<td>67345.9±26461.4</td>
<td>1429.7±741.6</td>
</tr>
<tr>
<td>Controls (n=111)</td>
<td>93.8±230.1</td>
<td>102.3±45.0</td>
<td>118944.1±99766.3</td>
<td>67345.9±26461.4</td>
<td>1429.7±741.6</td>
</tr>
<tr>
<td>Total (n=199)</td>
<td>87.1±230.1</td>
<td>135.2±225.7</td>
<td>177075.9±143942.1</td>
<td>66869.2±24841.7</td>
<td>1274.9±717.3</td>
</tr>
</tbody>
</table>
TIMP-1 expression. They found that TIMP-1 RNA is synthesized by astrocytes ringing MMP-elaborating inflammatory lesions. This suggests that TIMP-1 might function to contain further the MMP-mediated expansion of the inflammatory lesion by inhibiting tissue destruction caused by leukocyte migration [25].

Upregulation of MMP-2 in meningitis has not been reported [24]. Lee and others [26] showed that the persistent increase of MMP-2 concentrations in patients with tuberculous meningitis was associated with development of complications. Patients with late neurological complications had higher MMP-2 concentrations than those without neurological complications. In the current study, we did not find higher CSF MMP-2 concentrations in patients with meningitis in comparison with controls. Thus MMP-2 does not appear to play an important role in patients with meningitis.

Expression of TIMP-2 is mainly constitutive [27]. In our study, we did not find any significant differences in the levels of CSF TIMP-2 between patients with meningitis and controls. TIMP-2 is both an activator and an inhibitor of MMP-2 at lower and higher concentrations, respectively. TIMP-2 functions in connection with MT1-MMP to activate MMP-2 [28]. It is possible that either TIMP-2 does not play a major role in meningitis or selective sampling time points failed to reflect changes in MMP-2 or TIMP-2 expression in the CSF, because they are measured in specimens from patients beginning on the day they were hospitalized.

We found that the TIMP-4 concentration in CSF was substantially downregulated during the acute phase of meningitis. This change may be caused by alteration of the cytokine milieu and other biological factors that are major modulators of TIMP expression [29] and may reflect greater roles of these proteins in meningitis. The detailed mechanism for this process is still unknown.

There were limitations to our study. Our sample size for different meningitis aetiologies was relatively small. The sampling time was different because specimens were collected at the beginning of admission and patients were in different stages of illness. Also, we did not measure the serum/CSF albumin index. Although there were no significant differences in the serum concentrations of MMPs and TIMPs between patients with infectious meningitis and the controls, the possibility of MMP/TIMP accumulation in the CSF caused by passive influx instead of intrathecal production cannot be completely excluded.

In conclusion, we found that the MMP/TIMP system was dysregulated in patients with meningitis regardless of the aetiology of meningitis. Patients with bacterial meningitis tended to have higher CSF MMP-9 and TIMP-1 expression compared with controls and meningitis due to other causes.

Acknowledgements

This work was supported by the Summer Medical Student Research Program 2008, from the Medical Foundation in Memory of Dr Deh-Lin Cheng, grants VGHKS97-034 and VGHKS 98-039 from Kaohsiung Veterans General Hospital, and grants NSC-96-2320-B-075B-002 and 97-2320-B-075B-001-MY2 from the National Science Council, Republic of China.

Transparency Declaration

The authors have no conflicts of interest to declare.

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