Serum Levels of Matrix Metalloproteinases: Implications in Clinical Neurology

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Key Words

Matrix metalloproteinases • Multiple sclerosis • Myasthenia gravis • Stroke • Neurodegenerative disease

Abstract

Matrix metalloproteinases (MMPs) are zinc-dependent enzymes involved in remodeling extracellular matrix and cell-matrix interactions. A pathogenic role of MMPs in neurological disorders is likely. This paper focuses on serological clinical aspects only. In multiple sclerosis, higher serum MMP-3 is seen during relapses. Lower serum MMP-8 and -9 levels correlate with fewer contrast-enhanced T₂-weighted MRI lesions, and serum MMP-9 can be used in monitoring treatment. In myasthenia gravis, serum MMP-2, -3, and -9 levels are elevated in both generalized and ocular diseases. A proportion of the patients have markedly increased serum MMP-3. In acute stroke, higher serum MMP-9 correlates with larger infarct volume, stroke severity, and worse functional outcome, and serum MMP-3 is significantly lower than in several other neurological disorders and healthy controls. In amyotrophic lateral sclerosis, serum MMP-2 correlates with disease progression, and both serum MMP-1 and -2 are elevated. In Alzheimer's disease, serum MMP-3, -9, and -10 are elevated. In migraine, serum MMP-2 is elevated, and also

MMP-9 in those patients with migraine without aura. MMP-9 is implicated in the pathogenesis of experimental epilepsy. A pathogenic role of MMPs in these conditions could be related to their ability to degrade extracellular matrix. MMPs may also facilitate autoimmunity.

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Matrix Metalloproteinases

Matrix metalloproteinases (MMPs) represent a multi-member family of zinc-dependent proteases with a wide range of substrates, including extracellular matrix components, cytokines, receptors, and cell motility factors [1, 2]. They are recognized as the main proteolytic enzyme group involved in remodeling the extracellular matrix and modifying cell-cell and cell-matrix interactions [3]. MMPs play an important role in regulating organ development including muscles and nerves, maintaining normal physiology in adulthood, and they also take part in repair after injury in the nervous system.

Many different human MMPs have been identified [1]. Based on function and structure, MMPs can be subdivided into 5 groups: (i) collagenases (MMPs-1, -8 and -13); (ii) gelatinases A and B (MMPs-2 and -9); (iii) stromelysins-1

Fig. 1. Schematic representation of MMP-9 synthesis and activation. MMP-9 mRNA translocated toward the dendrites is locally translated with the protein eventually localized to secretory vesicles. In response to glutamate-driven neuronal activity, MMP-9 is released outside the cell where, through the cascade of proteolytic steps (involving, e.g., furin, MT1-MMP, MMP-2, tPA, uPA, and plasmin), it is transformed from the latent to the active form. Once activated, MMP-9 is quickly inactivated by interactions with its inhibitor TIMP-1. tPA = Tissue-type plasminogen activator; uPA = urokinase-type plasminogen activator; uPAR = uPA receptor. (From Michaluk and Kaczmarek [6], with permission from Nature Publishing Group.)

and -2 (MMPs-3 and -10); (iv) a more heterogeneous subgroup containing matrilysin (MMP-7), enamelysin (MMP-20), the *mmp20* gene product, macrophage metalloelastase (MMP-12), and MMP-19 (together making up the 'classical' MMPs), and (v) membrane-type MMPs (MT-MMPs-1–4 and stromelysin-3, MMP-11) [4]. Most MMPs are synthesized with a ~20 amino acid residue signal peptide and are secreted as pro-proteinases consisting of an ~80-residue N-terminal prodomain followed by the ~170-residue catalytic domain, which in turn is covalently connected through a 10- to 70-residue Pro-rich linker to a ~195-residue C-terminal hemopexin-like domain [4]. MMPs are expressed as inactive zymogens. The Zn^{2+} at the active site of the enzyme binds to the cysteine residue in the propeptide. When the interaction between cysteine and Zn^{2+} is disrupted, mainly by cleaving the propeptide, the proenzyme is activated [5, 6]. This activation is controlled by a series of steps involving other MMPs and the plasmin system, as demonstrated in MMP-9 (fig. 1) and MMP-2 (fig. 2). Free radicals, serine proteases, and other activated MMPs are among the factors that convert a pro-MMP to its active enzyme [1]. When activated, the MMPs can be inhibited by their tissue inhibitors (TIMPs) [6].

The TIMP family includes four different members (TIMPs-1–4), exhibiting 41–52% sequence identity [4, 7]. TIMPs have an inhibitory role and also may function as

growth factor-like agents. They may also show anti-angiogenic activity [8].

MMPs have catalytic properties due to the presence of Zn^{2+} . The catalytic domains of the MMPs exhibit the shape of an ellipsoid. A small active-site cleft harboring the catalytic Zn^{2+} is notched into the flat ellipsoid surface and extends horizontally across the domain to bind peptide substrates from left to right [4]. The degrading catalytic activity of MMPs is regulated by TIMPs, striking a balance that is necessary for normal biological function of these enzymes.

The gelatinase members of the MMP family, MMPs-2 and -9, have traditionally been the easiest to detect using gelatin zymography, and hence there is much more available data on them. Antibodies to both gelatinases and other MMP members have been developed making it even easier to detect MMPs by ELISA. RNA analyses are useful to ascertain MMP expression since primers are available for the majority of MMPs. Using real-time polymerase chain reaction, the majority of MMP members can be detected [1]. Most of these tests are commercially available and easy to use. Since MMPs may also be detected in some healthy individuals, the results from a patient group should always be compared to those from a group of healthy individuals where the cut-off values can be determined.

Fig. 2. Mechanism of pro-MMP-2 activation. **a** MT1-MMP on the cell surface acts as a receptor for TIMP-2. TIMP-2 binds via its N-terminal domain to the active site of the MT1-MMP. This binary complex then acts as a receptor for pro-MMP-2, with the TIMP-2 C-terminal domain binding to the C-terminal domain of pro-MMP-2. A free MT1-MMP molecule in close proximity can then cleave the propeptide of pro-MMP-2, generating an intermediate species. Further proteolysis of the propeptide through an autocatalytic mechanism results in the generation of the fully active enzyme. Activation of pro-MMP-2 in this model is only possible if the TIMP-2 concentration is low, with sufficient TIMP-2 to generate the trimolecular complex but not enough to saturate all the MT1-MMPs required for proteolysis of the propeptide. **b** At high levels, TIMP-2 will bind to and inhibit all MT1-MMP activity, preventing pro-MMP-2 activation. (From Expert Reviews in Molecular Medicine, with permission from Cambridge University Press.)

MMPs play an important role in normal function and pathology. In the central nervous system (CNS), for instance, MMP-9 is involved in the myelination process, and the lack of it in MMP-9-deficient mice leads to microcrania. MMPs-2 and -9 are involved in the repair process following CNS injury [1].

A balance between the degrading matrix MMPs and their TIMPs regulates the turnover of extracellular matrix in all solid organs [9]. A disruption of the MMP-TIMP balance may result in diseases such as rheumatoid arthritis (RA), osteoarthritis, atherosclerosis, tumor growth, metastasis, and fibrosis [4]. An altered extracellular matrix metabolism has been implicated in a variety of diseases, and the upregulation of MMPs in several neurological conditions has been associated with pathology [10, 11]. The production of MMPs is also influenced by genetic polymorphisms. In multiple sclerosis (MS), for instance, the genetic polymorphism of the MMP-9 promoter region was found to influence the susceptibility to MS [12]. A functional polymorphism in MMP-1 has been shown to alter the transcriptional activity of it, resulting in diverse pathological processes [13].

MMPs play a significant role in the pathogenesis of several autoimmune diseases such as RA, systemic lupus erythematosus (SLE) [14, 15], and Sjögren's syndrome [15]. An elevated serum MMP-9 level is found in SLE [16],

systemic sclerosis [17], RA [18], and Sjögren's syndrome [19]. Serum MMP-3 is increased in SLE and RA [14, 20]. A pathogenic role of MMPs in these conditions could be related to their ability to degrade extracellular matrix components, exposing self-antigens to the immune system and facilitating autoimmunity in general.

MMP inhibitors have been tested in the treatment of different acute and chronic conditions. MMP inhibitors usually act on the active MMP Zn^{2+} site and have different mechanisms of action. Some inhibitors contain hydroxamate that chelates the Zn^{2+} [21], while others contain carboxylic acids, thiols, or phosphorous-based structures [1].

In recent years, advances have been made regarding the role of MMPs in both autoimmune and non-autoimmune neurological disorders. The aim of this paper is to review this role, focusing on current and future clinical implications in selected neurological disorders for which clinical and serological MMP data are available.

Serum Levels of MMPs in MS

MS is an inflammatory autoimmune CNS disease with a lifetime risk of 1 in 400. The pathogenesis includes acute inflammatory injury of axons and glia, recovery of function and structural repair, postinflammatory gliosis, and

neurodegeneration. MS is mediated by autoreactive T lymphocytes with specificity for myelin antigens that trigger oligodendrocyte death associated with axonal loss and neurodegeneration in the CNS [22, 23]. The clinical course is characterized by episodes with recovery, episodes followed by neurological deficits, and secondary progression [22]. Genetic susceptibility is probably the most important determinant of MS pathogenesis, with HLA DRB1*1501 present in over 50% of northern MS patient populations. Environmental differences principally determine the regional variations in disease characteristics. Despite 75% of MS patients being women, men are more likely to be genetically susceptible [24]. Migration studies show that changes to early environment can greatly affect MS susceptibility [25]. Epigenetic modifications within the major histocompatibility complex are likely to interact with environmental risk factors, causing MS [26].

MMPs are involved in blood-brain barrier disruption and formation of MS lesions [27]. Several MMPs are elevated parallel to increased disease activity in experimental autoimmune encephalomyelitis (EAE) [28]. Increased MMP activity and reduced TIMP levels contribute to a loss of blood-brain barrier integrity. In a clinical MS trial, erythropoietin induced the expression of TIMP-1 in endothelial cells, which helped maintaining blood-brain barrier integrity. The protective effects of erythropoietin were associated with an increase in the number of astrocytes expressing TIMP-1 in the brain and spinal cord in EAE [29]. Decreased serum MMP-8 and MMP-9 levels correlated with a decreased number of contrast-enhanced T₂-weighted MRI lesions in MS patients [27]. In the same study, MS patients treated with IFN-beta1b showed a reduction in serum MMP-8 and MMP-9 in parallel with disease stabilization. The authors concluded that serial measurement of MMPs and other inflammatory mediators may serve as sensitive markers for therapeutic response to IFN-beta1b during the first year of treatment. Another study similarly suggested MMP-9 as a factor in MS treatment monitoring [30], as a twofold decrease in the serum MMP-9/TIMP-1 ratio correlated with decreased disease activity. Previously, it was demonstrated that IFN-beta was able to inhibit the production of MMPs by T lymphocytes [31].

Serum MMP-3 levels are higher during MS relapse than in remission [32]. This variation may be helpful in detecting disease activation. Stable MS patients have lower mean serum MMP-3 levels than myasthenia gravis (MG) patients and healthy controls [33]. Also, intrathecal MMP-2 production was increased in relapsing-remitting MS patients [34].

Extracellular matrix metalloproteinase inducer (EMMPRIN, CD147), a member of the Ig superfamily, is upregulated on peripheral leukocytes before the onset of EAE, which is an experimental model for MS. In EAE brain sections, EMMPRIN expression was localized with MMP-9 protein and activity. The increased EMMPRIN level was also characteristic of brain samples from MS subjects, particularly in plaque-containing areas. EMMPRIN regulates leukocyte trafficking through increasing MMP activity. EMMPRIN might therefore be a novel therapeutic target in MS [35].

The effect of intravenous IgG in MS is disputed, although it has been reported in an animal model that intravenous IgG might be effective as prophylaxis, preventing disease development, exerting its function by suppressing activation of MMPs-2 and -9 by blocking MMP activities at the interface between the blood stream and CNS [36].

Tetracyclines are known to inhibit MMPs-2 and -9, and have been used in both EAE and MS patients, resulting in promising reduction of relapse rates [37, 38]. Also MMP-8 has been reported to play a role in various inflammatory conditions such as MS. Therefore, it is interesting to develop MMP-8 selective inhibitors. One approach is immobilized enzyme reactors, allowing a simple way to determine affinity and thus the ranking of the inhibiting potency of the compounds under study. The immobilized enzyme reactor was successfully used for the screening of known MMP-8 inhibitors in zonal chromatography and inhibition experiments [39].

To summarize available clinical data on MMPs in MS in one single hypothesis on how MMPs play their role in MS (table 1), it is reasonable to assume that increased MMP activity enhances MS plaque formation and clinical deterioration through increased MMP ability to traverse the blood-brain barrier and promotion of neuroinflammation, followed by increased destructive catalytic activity inside the CNS resulting in demyelination and neurotoxicity [1, 10, 40]. It is not known whether this increased MMP activity is a causative primary plaque formation phenomenon in MS or a secondary result of other pathophysiological steps.

Serum Levels of MMPs in MG

MG is an autoimmune disorder which causes skeletal muscle weakness. Antibodies against the acetylcholine receptor (AChR) are present in 85% of patients with generalized MG (GMG) and in about 40% of patients with

Table 1. Summary of the action of the different MMPs in the various neurological conditions discussed in this review

Neurological disorder	MMPs involved	Serum level	Possible role	Possible future target of MMP inhibitor treatment
MS	3, 8, 9/TIMP-1 8, 9	elevated during relapses reduced after treatment	pathogenic markers of treatment response	yes
MG	2, 3, 9	elevated	pathogenic	yes
Stroke	3 9/TIMP-1 3	reduced in acute stroke elevated	pathogenic marker of worse outcome carotid atherosclerosis	unknown yes
ALS	1, 2, 9/TIMP-1	elevated	pathogenic	yes
AD	2, 3, 10	elevated	pathogenic	unknown
Migraine	2 9/TIMP-1	elevated elevated	pathogenic in migraine with aura pathogenic in migraine without aura	unknown unknown
Epilepsy	9		pathogenic	unknown

ocular MG (OMG) [41–43]. Most patients with OMG develop GMG during the first 2 years, but 15% remain with purely ocular symptoms for the entire course [42]. MG with AChR antibodies is known as seropositive MG (SPMG). Thymoma is present in 15% of MG patients [44]. AChR antibodies impair neuromuscular transmission by complement-mediated postsynaptic membrane damage, direct blockade of ligand-receptor interaction, and/or by cross-linking of AChR and increased degradation [41, 45]. Antibodies to muscle-specific kinase (MuSK) are observed in a minority of MG patients [46, 47]. MuSK is a key signaling protein controlling AChR clustering and the formation of the neuromuscular junction. Agrin interaction with MuSK leads to the clustering of AChR [48, 49]. MG without detectable antibodies to AChR and MuSK is termed seronegative MG (SNMG). Several other autoantibodies, against titin, ryanodine receptor, myosin, and α -actin, have been detected in sera from MG patients. These antibodies are associated with MG subtype and disease severity [50].

In an in vitro study, it was previously shown that MMP-9 is targeted by immunomodulated autoreactive T cells from MG-immunized mice [51]. Elevated serum MMP-3 levels are present in both SPMG and SNMG [52]. High serum MMP-3 levels were confirmed in another MG cohort, where the patients had OMG or MG with ocular muscle predominance [53]. Interestingly, in this cohort, females had significantly lower serum MMP-3 concentrations than males, and male patients were over-represented among those with higher serum MMP-3

concentrations (male:female ratio = 4:1). The elevated MMP-3 levels did not correlate with the presence of AChR antibodies, age of onset, or thymus pathology. MMP-3 concentration in the MG cohort was higher than in control autoimmune and non-immune neurological disorders [33]. Serum MMP-2 and MMP-9 concentrations were higher for both the GMG and OMG groups compared to healthy controls. The presence of MMP-2 did not correlate with the occurrence of AChR antibodies. No significant age or gender difference, nor difference between SPMG and SNMG patients, was observed for MMP-9.

The mechanism by which these MMPs exert their action in MG is not known (table 1). However, one could hypothesize about their role in regulating agrin levels through MMP-MMP interaction. We know that MMP-2 and MMP-3 are able to activate MMP-9 [54–57], whereas MMP-2 and MMP-9 are potent activators of MMP-3 [58]. Agrin is a substrate for MMP-3 [59]. MMP-3 null mice have alterations in their neuromuscular junctions, with increased AChR staining at the endplate, an increased number of junctional folds, and increased agrin concentration [60]. These observations indicate that MMP-3 controls the structure of the neuromuscular junction via regulation of agrin levels. Elevated MMP levels in MG may thus be involved in promoting autoimmunity, but also in the pathophysiological steps causing damage to the neuromuscular junction through increased MMP-2, -3, and -9 activity.

Serum Levels of MMPs in Acute Ischemic Stroke

Acute ischemic stroke is caused by blood vessel occlusion disrupting the blood supply to the brain causing tissue damage. Its treatment is time dependent and technically and logistically demanding [61]. Higher serum MMP-9 concentrations in the acute phase correlate with larger infarct volume, higher stroke severity, and worse functional outcome [62]. MMP-9 was also a predictor of intracerebral hemorrhage development in patients treated with thrombolytic therapy. In a cohort study of elderly men [9], serum MMP-9 and TIMP-1 levels correlated with the risk of all-cause mortality, while TIMP-1 levels were mainly related to cardiovascular mortality and stroke risk. The authors suggested MMP-9 and TIMP-1 levels as relevant markers for an altered extracellular matrix metabolism of clinical significance. It was previously demonstrated that several MMPs are increased in ischemic stroke [63].

In a group of consecutively collected acute stroke patients, none had elevated serum MMP-3 levels [33]. Mean serum MMP-3 concentration in the acute stroke group was reduced compared to healthy controls, and also to disease controls. MMP-3 may thus play a role in the pathophysiology of acute stroke. MMP-3 testing might therefore be useful as an indicator of acute cerebral ischemia with brain tissue damage in the very early phase where other diagnostic means, including MRI, are often inconclusive.

Studies have shown conflicting results regarding MMP-3 and blood vessel pathology. It is, however, possible to hypothesize on the mechanism by which MMPs are linked to cerebral ischemia and stroke (table 1). High serum MMP-3 levels have been linked to atherosclerosis in the carotid arteries [64] and to higher mortality following acute cerebral hemorrhage [65]. Low serum MMP-3 levels were seen in stroke patients with dolichoectasia [66]. Whether the low MMP-3 levels in acute stroke patients are primarily linked to the thromboembolic mechanisms that cause stroke or are a result of increased activation and consumption of MMP-3 in the tissue damage process following acute stroke is unknown. However, following cerebral ischemia and reperfusion in acute experimental stroke in rats, MMP-3 is activated causing cleavage of brain agrin as a step in the tissue damaging process [67]. This indicates increased activation and consumption of MMP-3 during the tissue damage immediately following an acute stroke. In experimental stroke, animals treated with MMP inhibitors 1 week after stroke had larger infarctions than those

treated 2 weeks after stroke [68], indicating that MMPs might play a pro-ischemic role during the early stages of stroke and a constructive role during the repair phase after stroke.

Serum Levels of MMPs in Other Neurological Disorders

In neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS) and Alzheimer's dementia (AD), MMPs may be useful as disease markers. In one study, serum MMP-2 concentration correlated with ALS progression [69]. In another study, serum MMP-1, MMP-2, MMP-9, and TIMP-1 were increased in mild ALS cases, while MMP-9 was decreased in cerebrospinal fluid (CSF) [70]. In the superoxide dismutase transgenic mouse model of ALS, MMP-9 deficiency improved both life expectancy and neuronal death [71]. Also in the same model, treatment with MMP inhibitors had the same beneficial results [72].

A recent study on AD found that MMP-3 was elevated in serum and CSF [73]. MMP-2 in CSF was significantly decreased, while levels in serum remained unchanged. MMP-9 was significantly decreased, whereas MMP-10 was unchanged in serum, and none of them could be detected in CSF.

A pathogenic role and a consistent diagnostic pattern of MMPs in neurodegenerative disorders remain to be established (table 1).

Migraine patients with and without aura have increased serum MMP-2 activity. The serum MMP-9/TIMP-1 ratio was higher in migraine without aura compared to migraine with aura, which may reflect pathophysiological differences between these two conditions [74] (table 1).

MMP-9 has been implicated in the pathogenesis of epilepsy and epileptic seizures in animal models, and also in experimental therapeutic approaches to epilepsy [75–77]. Any role of MMP-9 in clinical epileptology is still to be established. Serum MMP-3 concentration in epilepsy patients did not differ from that in healthy controls [33] (table 1).

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