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Matrix metalloproteinases and diseases of the central nervous system with a special emphasis on ischemic brain

Yvan Gasche [1, 2, 3], Paola Marina Soccal [3, 4], Michiko Kanemitsu [1], and Jean-Christophe Copin [1]

[1] Department of Fundamental Neuroscience, [2] Department of Anesthesiology, Pharmacology and Surgical Critical Care, [3] Department of Internal Medicine and [4]Department of Surgery, Geneva University Hospital and Geneva Medical School, CH-1211 Geneva, Switzerland

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1. ABSTRACT

Matrix metalloproteinases (MMPs) are involved in the pathogenesis of several diseases of the CNS, that share common pathophysiological processes, such as blood-brain barrier (BBB) disruption, oxidative stress, remodeling of the extracellular matrix (ECM) and inflammation. In ischemic brain injury, MMPs are implicated in various stages of the disease. Early after the onset of ischemia, MMPs contribute to the disruption of the BBB leading to vasogenic edema and to the influx of leucocytes into the CNS. The ability of MMPs to digest the basal lamina of capillaries increases the risk of hemorrhagic transformation of the ischemic tissue. During the acute ischemic phase, maintenance of the ECM is essential for neuronal survival. However, ECM degradation and its reconstitution are critical to tissue recovery. MMPs as a key modulator of ECM homeostasis play a role in the cascades leading to neuronal cell death and tissue regeneration. This pleiotropic implication of MMPs in brain injury has open new areas of investigation, which should lead to innovative therapeutic strategies. Yet MMPs may have a detrimental or beneficial role depending on the stage of brain injury. Simple therapeutic strategies based on MMP inhibition have thus little chance to favorably alter prognosis.

2. REGULATION OF MATRIX METALLOPROTEINASES (MMPs) IN THE CENTRAL NERVOUS SYSTEM

Matrix metalloproteinases (MMPs) belong to a family of Zn^{2+}- and Ca^{2+}-dependent endopeptidases including more than 20 members (1-3). Most MMPs are structurally organized into three domains; an aminoterminal propeptide, a catalytic domain and a hemopexin-like domain at the carboxy-terminal. In most cases, MMPs are secreted in the interstitial space as an inactive zymogen. A subclass of MMPs is membrane-bound (MT-MMP) and features a transmembrane domain and a short cytoplasmic C-terminal end or a hydrophobic region with a specific function (4). The regulation of MMP expression and activity appears to be a very complex and tightly controlled process. Tissue expression of most MMPs is low under normal conditions and is induced when remodeling of ECM is necessary. MMP gene expression is mostly regulated at the transcriptional level, but modulation of mRNA stability has been described for stromelysin, collagenase and gelatinase A (MMP-2) in response to growth factors and cytokines (5, 6) and for gelatinase B (MMP-9) in response to nitric oxide (NO) (7). The promoter gene sequence of several MMPs such as MMP-1, -3, -7, -9, -10, -12 and -13

contains an AP-1 site and an NF-κB site is identified on the promoter region of MMP-9 (8). Stimulation or repression of these growth factor-cytokine-redox responsive MMP genes results in major changes in mRNA and protein levels. On the other hand, the human gelatinase A promoter does not contain any cytokine responsive element but only an AP-2 site and behaves as a housekeeping promoter (9), leading to an extensive expression of MMP-2 under normal circumstances, with a low responsiveness to inflammatory stimuli.

What should be kept in mind, regarding MMP regulation, is that cells do not indiscriminately release these enzymes. MMPs are secreted and anchored to cell membranes acting thereby on specific substrate located in the peri-cellular space. For example, gelatinase A binds to the integrin avß3 (10), gelatinase B to CD44 (11), and matrilysin to surface proteoglycans (12). Since MMPs are secreted as latent enzymes, physiological or pathophysiological activation becomes a critical control point. The classic mechanism of MMP activation, "the cysteine switch mechanism", consists of the disruption of the interaction between a zinc molecule on the active site and a cysteine in the pro-domain, leading to the auto-proteolytic cleavage of the zymogen and the production of the mature active form of the enzyme (13). Chemical agents such as *p*-chloromercuribenzoate (14) or sodium dodecyl sulfate are able to generate active zymogen by exposing the zinc active site. Other agents such as reactive oxygen species or organomercurials inactivates the cysteine residue (15-17). Early description of factors in conditioned cell culture medium activating human proMMP-1 with no modification of the enzyme molecular weight (18) suggested that non-proteolytic mechanisms of MMP activation could exist in biological systems. In addition, S-nitrosylation or N-glycosylation of gelatinase B can also modify its activity (19, 20). Alternatively, proteolytic enzymes (14) may cleave the propeptide ahead of the cysteine, which will be removed afterwards autocatalytic digestion to produce a stable activated MMP. Among many proteases, plasmin and urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA) are known to serve as important physiological activators of MMPs (21). Metalloproteinases may also interact with each other and further promote activation as it has been described for MMP-3 or MMP-2 activation of gelatinase B (4, 22). In this regard, MMP-2 is activated by membrane-type MMPs (MT-MMPs). MT1-MMP, MT2-MMP, MT3-MMP, and MT4-MMP are expressed at low levels in many cell types. Although, MT1-MMP, which is regulated by cytokines, is the most predominant MT-MMP (23), both MT2-MMP and MT3-MMP share with MT1-MMP the ability to initiate the activation of pro-MMP-2. In contrast, MT4-MMP has no ability to process proMMP-2 into its active form. In addition to the activation of proMMP-2, MT1-MMP is responsible for the activation of proMMP-13 (15). Processing of proMMP-13 occurs via a 56 kDa intermediate and a final 48 kDa form.

After activation, MMP catalytic activity is regulated by the tissue inhibitors of metalloproteinases (TIMPs). Four TIMPs are identified so far: TIMP-1, -2, -3, and -4. They interact with the active site plus a site in the carboxyl terminal hemopexin-like region of MMPs. The C-terminal region of TIMPs interacts with the C-terminal region of the enzyme, increasing the rate of association many fold. TIMP-1, the natural inhibitor of MMP-9, prevents pro-MMP-9 activation. Furthermore, the pro-MMP-9-TIMP-1 complex can interact with MMP-3 and then dissociate into free pro-MMP-9 and the TIMP-1–MMP-3 complex. TIMP-2, the natural inhibitor of MMP-2, plays a dual role by also interacting with this gelatinase. Indeed, when TIMP-2 complexes with proMMP-2, cell surface-mediated activation of the enzyme is facilitated, whereas interaction of TIMP-2 with the active enzyme resulted in its inhibition (24-28).

3. MMPs IN THE ADULT BRAIN

The expression of MMPs is very low in the normal adult brain. MMP expression varies depending on the different brain regions, cell populations and state of development, as recently reviewed by Dzwonek et al (29). MMP-2 was identified in the cortex, cerebellum, hippocampus, mainly in astrocytes, but also in some neurons such as Purkinje cells or cortical neurons (30-33). MMP-9 was found in the cortex, cerebellum, and the hippocampus, while MMP-3 was present in the cerebellum (30-33). TIMPs were also found under normal conditions in different brain regions and cell types (29).

4. MMPs IN CNS PATHOLOGIES

4.1. Ischemic brain injury and multiple sclerosis

Experimental *in vivo* studies, investigating the role of MMPs in stroke, have shown that gelatinase expression was induced during experimental focal ischemia. Studies, conducted in spontaneously hypertensive rats resulted in some skepticism regarding the role of MMPs in the early phase of ischemic brain injury, since these studies could not show any up-regulation or activation of the enzymes during the first hours after permanent middle cerebral artery occlusion (MCAO) (34, 35). Later studies, using permanent or transient models of MCAO in mice, showed, however, that gelatinase B was already up-regulated 1 to 2 hours after ischemia (36, 37). Gelatinase B up-regulation was rapidly followed by the appearance of the active form of gelatinase B (37, 38). One study, conducted in baboons, showed an early up-regulation of gelatinase A (39). As a corollary to these experimental studies, in human ischemic stroke, MMP-9 expression was mainly increased in acute ischemic lesions (less than 1 week after stroke onset) while MMP-2 and MMP-7 (matrilysin) were increased in chronic lesions (more than 1 week after stroke onset) (40, 41). The oxidative unbalance induced by ischemia-reperfusion or mechanical brain injury was shown to be a key event in MMP-9 over-expression (42, 43).

As mentioned earlier, MMPs are expressed at low levels in resident cells of the normal brain. Under pathological conditions, the exact cellular source of the MMPs involved in BBB disruption is not known. Recently, Gidday *et al.* (44) identified recruited leucocytes, as a key

cellular source of MMP-9 in the early phase following experimental focal ischemia. Earlier studies (34) examined the expression of MMP-1 and MMP-3 (by western blotting) after permanent MCAO. These studies failed to show any expression of these proteins. Rosenberg *et al*. (45) could not find any MMP-3 immunostaining in normal cerebral cortex of spontaneously hypertensive rats, but they observed positive immunoreactivity for MMP-3 in activated microglia and ischemic neurons, 24h after reperfusion, using a model of transient MCAO (45). Inconsistent results were also found for TIMP-1 expression. While some studies showed an over-expression of TIMP-1 at the mRNA and protein level starting at 12 hours after the ischemic insult and reaching a peak at 48 hours (46, 47), others could not find any modulation of TIMP-1 expression during the first 24 hours (34, 37). On the other hand, Nguyen *et al.* (48) demonstrated that TIMP-1–independent activated MMP-9 was produced in endothelial cells, implying a more intricate intracellular regulation of the enzyme than previously thought (48).

The inconsistent results between studies observed regarding the expression of MMPs and TIMPs in brain ischemia may be related to methodological particularities of the experimental models, to variations in MMP measurement techniques and to species related specificities. In multiple sclerosis (MS), immunohistochemistry of brain tissue demonstrated that the expression of MMP-1, -2, -3, -7, -9 by astrocytes, microglia, endothelial cells and non resident cells such as T-cells and macrophages, was increased in and around plaques (40, 49, 50). During the development of experimental autoimmune encephalomyelitis (EAE), the increase of MMP-7 and -9 in brain tissue peaked at the time when clinical symptoms became evident (51). In human encephalitis, MMP-9 levels were increased in cerebrospinal fluid (CSF), whereas MMP-2, -3 and -7 levels were unaltered (52, 53). While MMP-9 CSF levels were similarly increased in MS patients with acute relapses and those in clinically stable phases of disease (53), MMP-9 serum levels appeared to increase during clinical relapses and high levels correlating with an increased number of gadolinium-enhancing MRI lesions (54) or with the risk to develop new gadolinium-enhancing lesions (55). Several studies have consistently shown that serum levels of MMP-9 were significantly higher in MS patients, compared to healthy control subjects (54-56). Accordingly, MMP-9 protein levels in peripheral blood mononuclear (PBMC) of active MS were significantly higher compared to controls (57). Furthermore, TIMP-1 was found to be up-regulated in chronic plaques (58), while concentrations of this inhibitor were low in CSF and plasma of MS patients (54, 57, 59-61); the level of TIMP-1 increased after treatment with interferon-beta (IFN-beta).

4.2. Traumatic brain injury

Over-expression of MMP-2 and MMP-9 has also been noted following traumatic and viral brain injury, as well as after kainate treatment (32, 62-64). MMP-9 was found to contribute to the pathophysiology of traumatic brain injury. In this regard, MMP-9 knockout mice were shown to have less motor deficits than wild-type mice after controlled cortical impact (64). At 7 days, traumatic brain lesion volumes on Nissl-stained histological sections were significantly smaller in MMP-9 deficient animals. In the course of kainate-induced seizure, early expression of MMP-9 (6-24h) was seen in the hippocampal dentate gyrus and neocortex. MMP-9 expression was observed in neuronal cell bodies as well as in dendritic layers (65). Several studies also showed that not only MMP-9 was increased, in the hippocampus following seizures, but TIMP-1 levels were increased as well (66, 67).

4.3. Bacterial infection

Bacterial meningitis is another example of inflammatory disease, where infectious organisms trigger a host inflammatory response. It is not surprising that up-regulation of MMPs was consistently observed in the course of bacterial meningitis (52, 68, 69). MMP-9 and MMP-8, were shown to be up-regulated in the CSF from children with bacterial meningitis, whereas MMP-2, -3 were not present (70). CSF levels of MMP-9 were significantly higher in patients who developed neurological sequelae than in those who recovered (70). CSF concentration of TIMP-1 increased with MMP-9, but remained elevated when the level of MMP-9 dropped. Experimental rat models of pneumococcal meningitis showed a dramatic induction of MMP-3, -8, -9, -12, -13, and -14, but not MMP-2 and -7, in brain parenchymal tissue. In cells found in the CSF, MMP-8 and -9 mRNA were significantly increased, while MMP-2 and -7 mRNA remained at basal levels (71, 72).

5. ROLE OF MMPs IN CNS PATHOPHYSIOLOGIC RESPONSES

MMPs are up-regulated in most of CNS pathologies inducing an inflammatory response. Since MMPs are not only key modulators of the ECM, but also critical effectors for the cleavage or shedding of various molecules (to their active or inactive forms) including pro-inflammatory cytokines such as IL-1beta (73) and Fas ligand (FasL) that are implicated in many inflammatory processes, MMPs appear to be involved at many different pathophysiologic levels in various brain pathologies.

5.1. MMPs in ischemic brain injury and disruption of the blood brain barrier

The main causes of early neurological deterioration and mortality following ischemic stroke are brain edema (74) and hemorrhagic transformation (75). The common pathophysiologic pathway leading to these complications is the disruption of the BBB. The role of BBB is to preserve the neuronal microenvironment, which is essential for cell survival and the normal function of the brain. BBB disruption is observed in experimental and clinical ischemic stroke (37, 76), after only a few hours following the ischemic event BBB disruption leads to vasogenic edema formation. Ischemia also rapidly triggers a pro-inflammatory cascade at the blood-vascular-parenchymal interface (77) that is further exacerbated by reperfusion (78). Free radical formation is an early and central event in the pathophysiology of brain ischemia-reperfusion (79). Among other stimuli, oxidative stress triggers proinflammatory cytokines such as TNFalpha and

IL-1beta, which were increased within a few hours of the insult (80); accordingly, post-reperfusion treatment with anti-TNFalpha neutralizing antibody reduced brain infarct volume and cerebral edema, as well as cerebral MMP-9 over-expression (81). These events signal vascular and leucocyte activation, leading to the appearance or increase of adhesion molecules for leucocytes on the surface of microvascular endothelial cells (82, 83) followed by endothelial-leucocyte adhesion and finally leucocyte penetration across the BBB into the ischemic tissue. Transmigrated leucocytes will further promote brain injury by releasing oxygen free radicals and various proteolytic enzymes. As a functional entity, BBB includes several cell types and the extracellular matrix (ECM). The endothelial basal lamina represents the non-cellular component of the BBB. Basement membrane proteins are synthesized by endothelial cells; the basal lamina is a specialized ECM composed of type IV collagen, fibronectin, laminin and various proteoglycans (84). The ECM components interact with endothelial cells *via* integrins and regulate distinct biological events such as cellular differentiation, survival, morphology, adhesion and gene expression (85-88). During cerebral ischemia-reperfusion, the ECM is disrupted. Major components of the endothelial basal lamina such as laminin, type IV collagen and fibronectin start to disappear as soon as 2 hours after the onset of ischemia (89). The first signs of BBB leakage were consistently observed between 2 to 8 hours after the onset of ischemia (37, 46, 90, 91). By 24 hours of experimental ischemia-reperfusion, dissolution of microvascular structures led to clear evidence of microvessel interruption (89, 92, 93) with local hemorrhage (92, 93). Thus, proteolysis seems to be a critical process of stroke related to BBB disruption.

All of the endothelial basal lamina components can be digested by MMPs. Among the MMPs, gelatinase A and gelatinase B specifically digest type IV collagen in the basal lamina. Rosenberg *et al.* (46) confirmed the role of MMPs in BBB disruption by demonstrating the ability of the non-selective MMP inhibitor BB-1101 (British Biotechnology, UK) to reduce early BBB leakage following transient focal ischemia. Asahi *et al.* (94) used knockout mice to demonstrate that among the MMPs, MMP-9 was the main MMP contributing to BBB disruption in experimental focal cerebral ischemia, while MMP-2 has no influence on brain injury (95). Recently, Fukuda *et al.* (96) formally demonstrated the role of activated proteases in vascular degradation.

The possible mechanism accounting for MMP-mediated BBB alteration is explained by the disruption of the endothelial basal lamina that prevents the anchorage of the endothelial cells onto the ECM. MMP-9 may also induce the loosening of the tight junctions by a the alteration of their constituents, such as ZO-1 (94). MMPs may also indirectly affect BBB permeability by interfering with inflammatory pathways triggered by ischemia-reperfusion. From *in vitro* studies, we know that MMPs can process IL-1beta into its biologically active form. While MMP-2 activated pro-IL-1beta in 24 hours, MMP-3 took 1 hour and MMP-9 only a few minutes to process the pro-IL-1beta into its active form. Similar observations were made for IL-8 (97). Thus, MMPs may promote inflammatory processes, and in a positive feedback loop increases MMP production by resident or migrating cells.

The fundamental role played by MMPs in the development of vasogenic edema during stroke is further substantiated by the fact that these enzymes mediate the capillary leakage triggered by oxidative stress. It is well established that the oxidative unbalance during focal cerebral ischemia is a major contributor to BBB disruption, secondary brain injury and hemorrhagic transformation (91, 98-100). During focal ischemia-reperfusion, mice deficient in Cu/Zn superoxide dismutase present with a more serious vasogenic edema that can be prevented by the inhibition of MMPs (42). MMP-mediated *in situ* proteolysis, observed at the capillary level, correlates with the local production of oxygen free radicals (42).

Microvascular disruption due to MMP proteolytic activity is a key event in ischemic stroke. This suggests that MMPs may be responsible for the increased risk of hemorrhagic transformation following recombinant tissue plasminogen activator (tPA) induced reperfusion. Recent experimental data confirmed this hypothesis. In a model of thromboembolic stroke followed by reperfusion with tPA in the rabbit, Lapchak *et al.* (101) showed that non-selective inhibition of MMPs, before the onset of ischemia, was able to reduce the hemorrhagic transformation. However, it is still unclear whether MMP inhibition during reperfusion would be efficient in reducing the hemorrhagic transformation after the ischemic event. Moreover, what would the optimal therapeutic window be? A first answer to this question was sought by Rosenberg et al. (102) who showed that a reduction in rat mortality occurred in animals subjected to MCAO followed by reperfusion with tPA and treated with an inhibitor of MMPs 2 hours after the ischemic event. Thus, accumulating data showed that MMP dysregulation was a major contributor to hemorrhagic transformation after reperfusion. In addition, recent studies suggested a close interaction between MMP and tPA pathways. Indeed, while exogenous tPA increased the ischemic level of MMP-9 in experimental stroke, tPA knockout mice showed decreased levels of MMP-9 (103, 104). On the other hand, it was also shown that an increase in endogenous tPA activity in the perivascular tissue following cerebral ischemia induced opening of the BBB by a mechanism that was independent of both plasminogen and MMP-9 (105). By contrast, Wang *et al*. (106) observed that MMP-9 played a detrimental role in a murine model of brain hemorrhage unrelated to ischemia.

One should underscore that clinical investigations resulted in observations that were in accordance with those made in experimental studies. Thus, in human ischemic stroke, basal MMP-9 plasma levels obtained before tPA treatment, appeared to predict intracranial hemorrhagic complications occurring after thrombolysis (107). On the other hand, a positive correlation was found between MMP-9 plasma levels and the infarct size (108, 109).

An additional point that was raised in an attempt to unravel the mechanism of BBB disruption and

hemorrhagic transformation was the issue of identifying the precise cellular source of gelatinase(s) implicated in vascular proteolysis. Although neurons (110), astrocytes (111), microglial cells (112), endothelial cells (34) and oligodendrocytes (114), express gelatinases, recent data suggested that MMP-9 secreted by transmigrated leucocytes was a major contributor to BBB disruption. To address this question, Gidday *et al.* (44) used MMP-9 knockout mice and chimeric knockout animals lacking either in leucocytes or in resident brain cell MMP-9 to determine if MMP-9 released from leucocytes and recruited into the brain during post-ischemic reperfusion, contributed to BBB disruption and brain injury. In this study, Gidday *et al*. (44) observed that the extent of BBB breakdown, the neurologic deficit, and the volume of infarction after transient focal ischemia were abrogated to a similar extent in MMP-9 knockout mice and in chimeras lacking leucocytic MMP-9, but not in chimeras with MMP-9-containing leucocytes. Zymography and western blot analysis of MMP-9 from these chimeras confirmed that the elevated MMP-9 expression in brain at 24 hours following reperfusion is derived largely from leucocytes. Interestingly, MMP-9 knockout mice showed a reduction in leucocyte-endothelial adherence and in the number of neutrophils plugging capillaries as well as infiltrating ischemic brain during reperfusion. Interestingly, microvessel immunoreactive collagen IV was also preserved in these animals (44). The results of these experiments reemphasized the role of leucocyte-derived MMPs in ischemia-related brain injury. The pathophysiologic complexity of leucocyte involvement in stroke combined with methodologic weaknesses in other studies probably explains the conflicting results obtained in human studies in which the efficacy of anti-leucocyte strategies were examined (114, 115). Finally, cortical spreading depression which is a propagating wave of neuronal and glial depolarization implicated in stroke, was also shown to alter BBB permeability by activating MMP-9 (116).

5.2. MMPs and TIMPs in ischemic brain injury and neuronal apoptosis

In response to internal or external death stimuli, mitochondria and other organelles may initiate apoptosis through the release of cytochrome c and activation of the intrinsic caspase pathway (117, 118,). Conversely, mitochondria may release apoptosis-inducing factor and initiate apoptosis by caspase-independent mechanisms (119, 120). Activation of cell surface receptors including Fas and TNFalpha receptor can also initiate apoptosis through the activation of caspase-8, which can activate the extrinsic caspase pathway by cleaving the proapototic molecule Bid that translocates to the mitochondria resulting in the release of cytochrome c (121, 122). Finally, oxidative DNA damage can trigger apoptosis by activating the transcription factor p53, up-regulating transcription of the Bax gene that encodes a pro-apoptotic protein with mitochondrial membrane permeabilization-inducing properties (123). Caspase-3 is the major downstream apoptosis effector enzyme. However, calpain, another cysteine protease, also appears to play a critical role in apoptosis, as judged by the anti-apoptotic effect of calpain inhibitors (124). Despite differences in cleavage-site specificity, both proteases cleave many common substrates, such as poly (ADP) ribose polymerase (PARP) and alpha-spectrin.

Brain ischemia-reperfusion triggers various pathophysiologic cascades, which interact in a global network with positive feed-back loops leading to neuronal cell death. The major participants to this network are oxidative stress and pro-inflammatory cytokines. In this context, several experimental studies have shown that strategies designed to reduce oxygen free radical formation (79) or pro-inflammatory cytokines (125) such as TNFalpha and IL-1beta release were efficacious in limiting neuronal injury. Oxidative stress was shown to promote the mitochondria-dependent (i.e. intrinsic) apoptosis pathway which is a major mechanism resulting in neuronal apoptosis in ischemia (126). MMPs, including MMP-2, MMP-3 and MMP-9, can convert the inactive precursor form of IL-1beta into biologically active forms, which are implicated in the development of brain damage following cerebral ischemia. MMP involvement in delayed cell death may also be related to the disruption of BBB leading to brain edema and secondary cell injury, but the recent observation that neuronal survival is closely related to the maintenance of the ECM (86), suggested a more direct role for MMPs in the pathophysiology of ischemic neuronal injury. Thus, Gu *et al.* (19) demonstrated that MMP-9 activation by S-nitrosylation induced neuronal apoptosis *in vitro*.

During cerebral ischemia *in vivo,* MMP-9 co-localized with neuronal nitric oxide synthase. Activated MMP-9 was identified, both *in vitro* and *in vivo,* as a stable sulfinic or sulfonic acid, whose formation was triggered by S-nitrosylation (19). Thus far only global ischemia and kainate models of brain injury have investigated the implication of MMPs in neuronal apoptosis *in vivo* or *ex vivo* (127, 128). In that regard, Lee *et al.* (127) showed that selective neuronal cell death in the hippocampus was reduced in MMP-9-deficient mice and also in animals treated with a non-selective inhibitor of MMPs when compared to control animals. However, the exact mechanism leading to the protection was not investigated. Jourquin *et al.* (128) also showed that non-selective inhibition of MMPs, as well as selective inhibition of MMP-9 were capable of protecting vulnerable neurons after kainate challenge to organotypic neuronal cultures.

Recent investigations conducted in our laboratory showed that non-selective MMP inhibition reduced cerebral infarct as well as DNA fragmentation after experimental focal ischemia. The cerebroprotective effect occurred concomitantly with a reduction of cytochrome c release into the cytosol, a reduction of calpain-related α-spectrin degradation, as well as an increase in the immunoreactivity of the intact form of poly (ADP) ribose polymerase (unpublished data). By contrast, specific targeting of the *mmp-9* gene in mice did not modify the apoptotic response after cerebral ischemia, although the intra-cerebroventricular injection of a non-selective inhibitor of MMPs in MMP-9 deficient mice provided a significant reduction of DNA degradation. These results indicated that

MMPs other than MMP-9 are actively involved in cerebral ischemia-induced apoptosis.

The involvement of TIMPs as a regulator of apoptosis was also studied. Glutamate-induced excitotoxicity was attenuated by TIMP-1 in cultured neurons (129). Interestingly, cytoprotection seemed to be independent of MMPs, since the nonselective inhibition of MMPs was unable to reproduce the cytoprotective effect produced by TIMP-1. TIMP-3 may also play a crucial role in ischemic neuronal cell death. Indeed, TIMP may stabilize the interaction between TNFalpha and its receptor as well as between FasL and Fas through the inhibition of MMPs since MMPS have protein- shedding abilities. While TIMP-3 is expressed at very low levels in normal brain tissue, TIMP-3 is highly expressed in ischemic cortical neurons undergoing apoptosis after experimental MCAO (45, 130). In addition, TIMP-3 and MMP-3 modified neuronal sensitivity to Fas-mediated apoptosis induced by doxorubicin (131). In this study, MMP inhibition by TIMP-3 appeared to be necessary for doxorubicin-induced apoptosis. MMP-3 added to cell cultures markedly attenuated apoptosis and blunted Fas receptor–FasL interactions at the neuronal cell surface (131).

5.3. MMPs in multiple sclerosis

Multiple slcerosis (MS) is an autoimmune disease characterized by demyelination and axonal loss. MMPs disrupt myelin (132) and fragments of the MMP mediated digestion of myelin basic protein (MBP) after injection into rodents can induce experimental autoimmune encephalomyelitis (EAE), the experimental model of MS, (133, 134). Also, human MMP-9 cleaves human MBP into peptide fragments, one of which contains the immunodominant epitope (134). Non-selective inhibitors of MMPs improved or prevented EAE (2). Moreover, young mice lacking MMP-9 have less symptoms after the induction of EAE when compared with wild type animals (135). In humans, IFN-beta inhibited the production of MMP-9 by leucocytes *in vitro*, and alters the capacity of leucocytes to cross endothelial (136) and ECM barriers (137, 138). In addition, MS patients treated with IFN-beta showed a decrease in serum MMP-9 as well as the number of leucocytes producing the proteinase (139). Interestingly, activated MMP-9 is capable of degrading and inactivating IFN-beta (140). IFN-beta degradation can be inhibited by minocycline, an antibiotic of the tetracycline family, known to inhibit MMPs and to protect against experimental focal ischemia (140, 141).

Other MMPs including MMP-12 also appear to play a critical role in MS, as macrophages from MMP-12 deficient mice showed a diminished capacity to penetrate basement membranes *in vitro* and *in vivo* (142). As in the case of ischemic brain injury the ability of MMPs to shed critical pro-inflammatory cytokines, adhesion molecules, TNFalpha and Fas receptors is a potential mechanism implicating MMP involvement in MS pathophysiology.

5.4. MMPs in bacterial meningitis.

We have previously discussed experimental models of bacterial meningitis. Despite progress in intensive care and effective antimicrobial chemotherapy, bacterial meningitis in man is still associated with a high mortality and incidence of neurological sequelae (143, 144). Following pneumococcal autolysis, a rapid increase of pro-inflammatory cytokines (IL-1beta, TNFalpha, IL-6) and chemokines (IL-8, MIP1-2) is detected in CSF, followed by increased BBB permeability (145). Over the past few years, experimental studies provided converging evidence for a central role of MMPs in bacterial meningitis (70-72, 146-150). Indeed, Leib et al. (71, 72) showed that an association between a broad-spectrum inhibitor of MMPs and antibiotics reduced neuronal necrosis and apoptosis in a model of experimental meningitis. The important results of these studies were a significant reduction in mortality, seizure incidence and a preservation of learning capacity in animals treated with the MMP inhibitor. The results of the studies by Leib *et al.* (71, 72) were, however, challenged by Bottcher *et al*. (151) who showed that MMP-9-deficient mice infected with streptococcus pneumoniae were not protected when compared to wild type animals, probably due to a delayed bacterial blood clearance. This result could be explained by the fact that the animals in this study did not receive any antibiotic following bacterial CNS infection.

5.5. MMPs and other CNS pathologies

MMPs are also associated with Alzheimer's disease, inflammatory myopathies and tumors of the CNS such as glioma (2, 3, 152). It is also likely that the number of neurological diseases associated with unbalanced MMP/TIMP expression will grow over time.

6. PERSPECTIVE

MMPs play a pleiotropic role in the pathophysiology of BBB disruption and delayed neuronal cell death during ischemic and inflammatory brain injury. Recent studies also suggest that MMPs may play a role in glial scarring, neuronal cell migration and brain tissue recovery. In this context, new therapeutic strategies designed to modulate MMP activity should take into account the multiple levels at which these proteinases act as well as the timing of their involvement in different pathophysiologic processes. An oversimplified experimental approach to new therapeutic interventions using MMP inhibitors would almost certainly bring about disappointing clinical results. In this regard, clinical stroke studies have been most often deceiving. Future studies should dissociate the acute phase of stroke, where MMPs play a deleterious role on the BBB and neuronal cell survival, from the sub-acute phase, when MMPs may play a more beneficial role by favoring neuronal cell migration and recovery.

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Send correspondence to: Yvan Gasche, MD, Surgical & Medical Intensive Care Geneva University Hospital 24, Rue Micheli-du-Crest, CH-1211, Geneva, Switzerland, Tel: 41/22-372-7440, Fax: 41/22-372-7455, E-mail: yvan.gasche@medecine.unige.ch

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