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Matrix Metalloproteinase-3 Promotes Early Blood–Spinal Cord Barrier Disruption and Hemorrhage and Impairs Long-Term Neurological Recovery after Spinal Cord Injury

Jee Youn Lee,*† Hae Young Choi,*† Hyun-Jong Ahn,‡ Bong Gun Ju,§ and Tae Young Yune*†¶

From the Age-Related and Brain Diseases Research Center, the Neurodegeneration Control Research Center,† and the Departments of Microbiology‡ and Biochemistry and Molecular Biology,¶ School of Medicine, Kyung Hee University, Seoul; and the Department of Life Science,§ Sogang University, Seoul, Republic of Korea*

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Address correspondence to Tae Young Yune, Ph.D., Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Medical Bldg 10th Floor, Dongdaemun-gu, Hoegi-dong 1, Seoul 130-701, Republic of Korea. E-mail: tyune@khu.ac.kr.

After spinal cord injury (SCI), blood–spinal cord barrier (BSCB) disruption by matrix metalloproteinases (MMPs) leads to BSCB permeability and blood cell infiltration, contributing to permanent neurological disability. Herein, we report that MMP-3 plays a critical role in BSCB disruption after SCI in mice. MMP-3 was induced in infiltrated neutrophils and blood vessels after SCI, and NF-κB as a transcription factor was involved in MMP-3 expression. BSCB permeability and blood cell infiltration after injury were more reduced in *Mmp3* knockout (KO) mice than in wild-type (WT) mice, which was significantly inhibited by *Mmp*3 siRNA or a general inhibitor of MMPs, N-isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid. The level of tight junction proteins, such as occludin and zonula occludens-1, which decreased after SCI, was also higher in *Mmp3* KO than in WT mice. Exogenously, MMP-3 injection into the normal spinal cord also induced BSCB permeability. Furthermore, MMP-9 activation after injury was mediated by MMP-3 activation. Finally, improved functional recovery was observed in *Mmp3* KO mice compared with WT mice after injury. These results demonstrated the role of MMP-3 in BSCB disruption after SCI for the first time and suggest that the regulation of MMP-3 can be considered a therapeutic target to inhibit BSCB disruption and hemorrhage, and thereby enhance functional recovery after acute SCI. *(Am J Pathol 2014, 184: 2985–3000; http://dx.doi.org/10.1016/j.ajpath.2014.07.016)*

Traumatic spinal cord injury (SCI) is a devastating condition that results in permanent disability. Currently, treatment options are limited, but significant advances have been made in understanding the pathophysiological features of SCI. Initial mechanical injury, followed by secondary injury, is known to contribute to pathophysiological features, leading to cell death and functional disability after SCI.[1]

The blood–spinal cord barrier (BSCB) is the functional equivalent of the blood-brain barrier (BBB), providing a specialized microenvironment for the cellular constituents of the spinal cord. The barrier function of BSCB is based on the specialized system of nonfenestrated endothelial cells and their accessory structures, including basement membrane, pericytes, and astrocytic end feet processes, which provide its regulatory and protective functions.[2] When BSCB is damaged by injury,

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blood cells cross into injured parenchyma and contribute to secondary injuries, such as inflammation.[3–5] These secondary damages induce apoptosis of neurons and glia, leading to permanent neurological deficits.[6]

Matrix metalloproteinases (MMPs) are known to degrade extracellular matrix and other extracellular proteins[7] and are essential for remodeling of extracellular matrix and wound healing.[8] However, excessive proteolytic activity of MMPs can be detrimental, leading to numerous pathological conditions, including BBB/BSCB disruption after injury.[6,9–12] For example, MMP-9 plays a key role in abnormal vascular permeability after SCI, and blocking of MMP-9 improves functional recovery.[6] After SCI, MMP-12 up-regulation increases BSCB permeability, followed by blood cell infiltration, thereby hindering recovery of motor function.[13] After brain focal ischemia, the degradation of tight junction (TJ) proteins is also blocked by inhibiting MMP-2 and MMP-9 activities.[14]

Several studies suggest that MMP-3 is involved in BBB disruption after injury. For example, lipopolysaccharide (LPS)–induced BBB disruption is reduced in *Mmp3* knockout (KO) mice when compared with wild-type (WT) mice.[11] In addition, cyclooxygenase-1 and cyclooxygenase-2 modulate LPS-induced BBB disruption through MMP-3 and MMP-9.[15] Recently, BBB disruption in a MPTP mouse model of Parkinson's disease is suppressed by MMP-3 deletion.[16] However, no direct evidence for the role of MMP-3 in the BBB/BSCB disruption after accidental trauma, such as SCI and/or ischemic stroke, has been presented yet. Thus, we examined the precise role of MMP-3 in BSCB disruption after SCI through four different approaches: i) *Mmp3* KO mice, ii) *Mmp3* siRNA, iii) a general inhibitor of MMPs, and iv) exogenous MMP-3 injection. Finally, we determined the effect of MMP-3 on functional recovery and histological outcome after SCI by comparing *Mmp3* KO mice with WT control.

Materials and Methods

Animals and SCI

We used adult (13- to 16-week-old, 28 to 30 g) male WT ($n = 171$), *Mmp3* KO ($n = 60$), and $Ikk\beta^{\Delta mye}$ ($n = 21$) mice in this study. *Mmp3* KO mice were generously donated by Dr. Tong Hyup Joh (Cornell University, Ithaca, NY). Myeloid cell type–specific IκB kinase (Ikk)-β–deficient ($Ikk\beta^{\Delta mye}$) mice were donated by Dr. Sung Joong Lee (Seoul University, Seoul, Republic of Korea). Because the genetic background of these mice has been maintained in C57BL/6, the same strain from Samtako (Osan, Republic of Korea) was used as WT. Adult male mice (18 to 22 g; Samtako) were anesthetized with 4% chloral hydrate, and a laminectomy was performed at the T9 to T10 level, exposing the cord beneath without disrupting the dura. The exposed dorsal surface of the cord then was subjected to moderate contusion injury (50 kilodyne force per 500- to 600-μm displacement) using the Infinite Horizons impactor (Infinite Horizons Inc., Lexington, KY). For sham–operated on controls, animals underwent a T9 to T10 laminectomy without contusion injury. Surgical interventions and postoperative animal care were performed in accordance with the Guidelines and Policies for Rodent Survival Surgery provided by the Animal Care Committee of the Kyung Hee University (Seoul, Republic of Korea).

Drug Administration

Mouse *Mmp3* siRNA and control nontargeting siRNA (control siRNA) were purchased from Thermo Fisher Scientific (Lafayette, CO). Each targeted siRNA was a mixture of four siRNAs formulated to enhance the effectiveness of the mixture in knocking down the target gene. The control siRNA was a mixture of siRNAs with scrambled sequences, confirmed by microarray to have minimal targeting of known genes in human, mouse, and rat cells. siRNAs were dissolved in distilled water (pH 7.4) and injected bilaterally (2 μL per site; final, 0.01 or 0.05 nmol per cord) into the spinal cord at 30 minutes after SCI, as previously reported.[17] An active MMP-3 recombinant enzyme (Calbiochem, La Jolla, CA) was dissolved in phosphate-buffered saline (PBS) and then injected bilaterally (5 μL per site; final, 20 or 200 pmol per cord) in normal spinal cord. Bilateral intraspinal injection was performed at the T9 to T10 level, according to the following stereotaxic coordinate: lateral ± 0.3 mm, 0.5-mm depth. Control groups received injections of equal volumes of control siRNA or PBS. A general inhibitor of MMPs, N-isobutyl-N-(4-methoxyphenylsulfonyl)glycyl hydroxamic acid (NNGH; Enzo Life Sciences International, Plymouth Meeting, PA) was dissolved in 10% dimethyl sulfoxide (5 μL of 5 mmol/L NNGH) and administrated by intraspinal injection (lesion center, 0.5-mm depth) using glass pipette at 30 minutes after SCI, as previously described.[18] Intraspinal injection into the spinal cord was performed using a pulled glass capillary pipette (30-μm external tip diameter), as previously described.[19]

Tissue Preparation

Tissue preparation was performed as previously described.[20] At specific time points after SCI, animals were anesthetized with chloral hydrate (500 mg/kg) and perfused via cardiac puncture initially with 0.1 mol/L PBS, pH 7.4, and subsequently with 4% paraformaldehyde in 0.1 mol/L PBS, pH 7.4. A 20-mm section of the spinal cord, centered at the lesion site, was dissected out, post-fixed by immersion in the same fixative for 4 hours, and placed in 30% sucrose in 0.1 mol/L PBS, pH 7.4. The segment was embedded in optimal cutting temperature compound for frozen sections, and longitudinal or transverse sections were then cut at 10 or 20 μm. For molecular and biochemical works, animals were perfused with 0.1 mol/L PBS, and segments of spinal cord (8 mm), including the lesion site, were isolated and frozen at −80°C.