Neurochemistry International 96 (2016) 46–55

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Distinct roles for metalloproteinases during traumatic brain injury

Si Zhang [a], Luba Kojic [a], Michelle Tsang [a], Parampal Grewal [a], Jie Liu [b], Dhananjay Namjoshi [c], Cheryl L. Wellington [c], Wolfram Tetzlaff [b], Max S. Cynader [a], William Jia [a, *]

[a] Brain Research Center, University of British Columbia, Vancouver, BC, Canada
[b] International Collaboration on Repair Discoveries (ICORD), University of British Columbia, Vancouver, BC, Canada
[c] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada

ARTICLE INFO

Article history:
Received 19 August 2015
Received in revised form
16 February 2016
Accepted 25 February 2016
Available online 3 March 2016

Keywords:
Traumatic brain injury
Closed and open head injury
Mice model
Metalloproteinases
Gelatinase activity
Immunohistochemistry

ABSTRACT

Background: Significant protease activations have been reported after traumatic brain injury (TBI). These proteases are responsible for cleavage of transmembrane proteins in neurons, glial, and endothelial cells and this results in the release of their extracellular domains (ectodomains).
Methods: Two TBI models were employed here, representing both closed head injury (CHI) and open head injury (OHI). *In situ* zymography, immunohistochemistry, bright field and confocal microscopy, quantification of immunopositive cells and statistical analysis were applied.
Results: We found, using *in situ* zymography, that gelatinase activity of matrix metalloproteinases (MMP)-2 and MMP-9 was upregulated in cortex of both injury models. Using immunohistochemistry for several MPPs (Matrix metalloproteinases) and ADAMs (disintegrin and metalloproteinases), including MMP-2, -9, ADAM-10, -17, distinct patterns of induction were observed in the two TBI models. In closed head injury, an early increase in protein expression of MMP-2, -9 and ADAM-17 was found as early as 10 min post injury in cortex and peaked at 1 h for all 4 proteases examined. In contrast, after OHI the maximal expression was observed locally neighboring the impact site, at a later time-point, as long as 24 h after the injury for MMP-2 and MMP-9. Confocal microscopy revealed colocalization of the 4 proteases with the neuronal marker NeuN in CHI, but only MMP2 colocalized with NeuN in OHI.
Conclusions: The findings may lead to a trauma-induced therapeutic strategy triggered soon after a primary insult to improve survival and to reduce brain damage following TBI.

1. Background

Traumatic brain injury (TBI) is a leading cause of mortality and morbidity among young adults and children in the developed world. After traumatic brain injury the initial primary mechanical tissue damage quickly triggers a complex cascade of secondary injury, including vascular, metabolic, cellular, and molecular processes that exacerbate damage, limit recovery, and contribute to overall morbidity and mortality (Reilly, 2001). The secondary mechanisms of injury including oxidative stress, inflammatory response, and excitotoxicity lead to activation of several matrix metalloproteinases (Gold et al., 2009; Lo et al., 2002; Pineda et al., 2004; Siao and Tsirka, 2002; Vilalta et al., 2008b; Yong, 2005) that further exacerbate the injury resulting in opening of the blood–-brain barrier (BBB) (Cunningham et al., 2005), prevention of normal cell signaling, and eventually leading to cell death (Shlosberg et al., 2010). Current management, focused on preventing secondary brain injury, has demonstrated significant progress in recent decades leading to reduced mortality. Halting the evolution of the primary injury is a critical goal for management of TBI.

Matrix metalloproteinases (MMPs) and the disintegrin and metalloproteinases (ADAMs) are families of zinc-binding proteolytic enzymes, and represent major regulatory systems in the brain. They normally remodel the extracellular matrix and play important roles in the development of the nervous system, regulating

* Corresponding author. Neurosurgery Division, Department of Surgery, Faculty of Medicine, University of British Columbia, 2211 Wesbrook Mall, Vancouver V6T 2B5, Canada.
E-mail addresses: zhang.si@hotmail.com (S. Zhang), lkojic@mail.ubc.ca (L. Kojic), myt1@sfu.ca (M. Tsang), parampal.grewal@gmail.com (P. Grewal), jliu@icord.org (J. Liu), dnamjoshi@cmmt.ubc.ca (D. Namjoshi), cheryl@cmmt.ubc.ca (C.L. Wellington), tetzlaff@icord.org (W. Tetzlaff), cynader@brain.ubc.ca (M.S. Cynader), w.jia@ubc.ca (W. Jia).

http://dx.doi.org/10.1016/j.neuint.2016.02.013
0197-0186/

proliferation, migration, differentiation and survival of various cells, as well as axonal growth and myelination (Yang et al., 2006).They also pathologically degrade substrates as part of the neuroinflammatory response during ischemia (Cunningham et al., 2005) and neurodegeneration (Rosenberg, 2009). By cleaving their substrates, metalloproteinases are responsible for shedding of transmembrane proteins in neurons, glial, and endothelial cells and the release of their extracellular domains (ectodomains) (Peschon et al., 1998). The injury-induced neuroinflammatory response increases the activity of metalloproteinases. Several key MMPs and ADAMs have been implicated in neuroinflammation after brain injury including MMP-2 and MMP-9 (gelatinases A and B), MMP-3 (stromelysin-1), membrane-type MMPs (MT1-MMP or MMP-14), ADAM-17 also known as tumor necrosis factor-α (TNFα) converting enzyme (TACE), and tissue inhibitor of metalloproteinases TIMP-3 (Candelario-Jalil et al., 2009; Grossetete and Rosenberg, 2008; Vilalta et al., 2008b; Walker and Rosenberg, 2009; Xue et al., 2009).

MMP-2 and MMP-9 are secreted as latent enzymes and are involved in diverse homeostatic and pathological processes (Cunningham et al., 2005; Rosenberg, 2009; Yong, 2005). Both require activation by the proconvertase furin, itself activated by HIF1α, a hypoxia-induced transcription factor. MMP-2 is constitutively present in large quantities in normal astrocytes and CSF. Since it is tethered to the cell surface, MMP-2 demonstrates space-restricted proteolytic activity, only in the vicinity of the membrane. On the other hand, MMP-9 is inducible and is released into the extracellular space without space—constraints, to degrade multiple proteins in the extracellular matrix (ECM) surrounding neurons (Cunningham et al., 2005; Rosenberg, 2009; Yong, 2005).

ADAMs are transmembrane proteins that mediate intercellular signaling by binding to integrins, but are also important for intracellular signaling and cell adhesion (Blobel, 2000). Although more than thirty mammalian ADAMs have been identified so far, only 17 are known to have some role in the brain (Rosenberg, 2009; Yang et al., 2006). ADAM-10 (Kuzbanian) and ADAM-17 (also known as TNF-α converting enzyme TACE) are membrane bound proteases expressed in cerebral and cerebellar cortex, hippocampus, and hypothalamus, in neurons, endothelial cells and astrocytes (Goddard et al., 2001; Skovronsky et al., 2001). In addition to their role in APP α-secretase activity (Asai et al., 2003; Buxbaum et al., 1998; Postina, 2008), both cleave many substrates including pro-TNFα, Notch, type-IV collagen, prion precursor, CD44, ephrins, cell adhesion molecule NCAM-L1, EGFR ligand, IL-1 receptor II, IL-6 receptor, L-selectin, type-XVII collagen, and chemokine fractalkine (Ludwig et al., 2005). The expression and activity of ADAM-17 increases under pathological conditions such as stroke and traumatic brain injury. It also promotes neural progenitor cell migration and contributes to stroke-induced neurogenesis (Katakowski et al., 2007).

A growing body of evidence suggests that metalloproteinases are markedly upregulated in the brain in response to injury and are responsible for the propagation and regulation of neuroinflammatory processes that accompany many forms of CNS disease (Cunningham et al., 2005; Rosenberg, 2009; Yong et al., 2007b). However, information regarding their activation in the brain following traumatic brain injury is lacking. The first aim of this study was to examine the expression and activation pattern of various metalloproteinases after traumatic brain injury. Information on the activation of particular proteases shortly after a brain injury is required for the development of a new type of gene delivery vehicle that we are currently developing. This consists of a membrane-bound construct, embodying a specific cleavage site and a therapeutic amino acid sequence that can be released by specific proteases following neurotrauma. This type of construct could have powerful protective potential, since it would spring into action rapidly after the traumatic insult even if the victim were alone or far from a medical facility. Our lab is in the process of developing such a trauma-inducible gene delivery system (Zhang et al., 2012, 2013) and understanding the pattern of protease activity following neurotrauma is an important step along this developmental path.

2. Material and methods

2.1. Traumatic brain injury

All procedures were approved by the Animal Ethics Committee (AEC) of the University of British Columbia, BC, Canada.

2.2. Closed head injury

A closed head injury (CHI) model of TBI (Flierl et al., 2009) was employed to assess the expression and activation of proteases induced immediately after traumatic brain injury. The CHI is a model of concussive and diffuse brain injury. This type of injury is difficult to reproduce with fluid percussion, controlled cortical impact, or focal brain contusion models that are more often associated with focal axonal damage. This model induces a CHI using a standardized weight-drop device inducing a focal blunt injury over an intact skull without pre-injury manipulations. Furthermore, the CHI weight drop TBI model triggers a profound neuroinflammatory response leading to neurological impairment and breakdown of the blood—brain barrier. As a result, the CHI model can induce early brain edema followed by both apoptotic and necrotic neuronal cell death.

Adult male C57Bl6 mice (Center for Disease Modeling, UBC) were housed under controlled environmental conditions with ambient temperature of 22 °C, relative humidity of 65% and 12 h light/dark cycle, with free access to food and water. All surgical procedures were conducted using aseptic techniques and animals were kept warm using a heating pad. Adult (8—10 weeks old) C57Bl6 mice were anesthetized with isoflurane (induction: 3—4%, maintenance: 1.5—2%) in oxygen (0.9 L/min) delivered through a nose-cone. A surgical plane of anesthesia was maintained throughout the surgery, confirmed by testing for loss of papillary and corneal reflexes as well as loss of toe-pinch reflex. Lubricating eye ointment was applied to prevent corneal drying. Ketamine (15 mg/kg) and xylazine (1.75 mg/kg) mixture (both s.c.), meloxicam (1 mg/kg, s.c.), and bupivacaine (0.125 mg/kg, s.c., under the scalp) were administered for analgesia. Sterile, warm saline (1 ml/100 g body weight) was administered s.c. to prevent dehydration.The skin overlying the head of the animal was shaved and a midline longitudinal incision in the skin was performed. The skin was gently retracted to expose the calvarium. An area ~2 mm lateral to the sagittal suture and ~2 mm posterior to the coronal suture in the left parietal bone of the head was marked for injury. Anesthesia was momentarily discontinued, the animal was quickly moved under the weight-drop device, and a 95 g weight was dropped on the skull from a height of about 6 cm to induce a moderate trauma resulting in a focal injury to the left hemisphere.The tip of the Teflon-tipped cone of the injury device has a tip diameter of 2 mm. The precise drop height (cm) of the weight was adjusted to be one-third the weight (g) of the animal. Mean body weight and drop height did not differ significantly across the sham and injured groups. Anesthesia was reinstated following injury. The incision was closed and the animals were kept in a recovery chamber with a heating pad until fully recovered. They were then returned to their cages. Mice that sustained skull fractures were immediately euthanized. Sham controls received isoflurane anesthesia, pre-