Neurobiology of Aging xxx (2015) 1–9

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Matrix metalloproteinase 9–mediated intracerebral hemorrhage induced by cerebral amyloid angiopathy

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ARTICLE INFO

Article history:
Received 15 May 2014
Received in revised form 1 July 2015
Accepted 8 July 2015

Keywords:
Cerebral amyloid angiopathy (CAA)
Matrix metalloproteinase (MMP)
Intracerebral hemorrhage
Multiphoton microscopy
Amyloid-β

ABSTRACT

Cerebral amyloid angiopathy (CAA), the deposition of amyloid-β in cerebrovascular walls, is the most common cause of lobar hemorrhagic stroke. Previous studies show that cerebrovascular amyloid-β induces expression and activation of matrix metalloproteinase 9 (MMP-9) in cerebral vessels of amyloid precursor protein transgenic mice. Here, we extended these findings and evaluated MMP-9 expression in postmortem brain tissues of human CAA cases. MMP-9 colocalized with CAA, correlated with the severity of the vascular pathology, and was detected in proximity to microbleeds. We characterized a novel assay using longitudinal multiphoton microscopy and a novel tracer to visualize and quantify the magnitude and kinetics of hemorrhages in three dimensions in living mouse brains. We demonstrated that topical application of recombinant MMP-9 resulted in a time- and dose-dependent cerebral hemorrhage. Amyloid precursor protein mice with significant CAA developed more extensive hemorrhages which also appeared sooner after exposure to MMP-9. Our data suggest an important role for MMP-9 in development of hemorrhages in the setting of CAA. Inhibition of MMP-9 may present a preventive strategy for CAA-associated hemorrhage.

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1. Introduction

Cerebral amyloid angiopathy (CAA) is the deposition of amyloid-β (Aβ) primarily in cerebral arteries and is present in 10%–40% of general autopsies and ≥80% of cases of Alzheimer's disease (AD) (Glenner and Wong, 1984; Jellinger, 2002; Okazaki et al., 1979; Vinters, 1987). CAA is often found accompanied with lobar hemorrhages and cerebral infarcts and is believed to be the most common cause of lobar hemorrhagic stroke (Greenberg, 1998; Greenberg and Vonsattel, 1997; Jellinger, 2002; Mandybur, 1986; Okazaki et al., 1979; Vonsattel et al., 1991). Unlike nearly all other causes of stroke, little is known about the molecular pathogenesis of CAA and currently no preventive treatment is available. Accumulation of Aβ in vessel walls induces many vasculopathic abnormalities, such as vessel wall thickening, luminal narrowing, hypoperfusion, vascular smooth muscle cells degeneration, and inflammation (Cadavid et al., 2000; Greenberg, 1998; Greenberg and Vonsattel, 1997; Jellinger, 2002; Mandybur, 1986; Vinters, 1987; Vonsattel et al., 1991). However, the deposition of Aβ is not sufficient for destruction of cerebrovascular integrity, which suggests that additional destructive mechanisms are likely involved in the basement membrane breakdown or the injury of the vessel walls.

One class of molecules that may participate in the regulation of vascular integrity are the matrix metalloproteinases (MMPs). MMPs are a family of 23 zinc-dependent endopeptidases capable of degrading virtually all the components constituting basement membranes and connective tissue. MMPs are therefore assumed to play an essential role in the homeostasis of the extracellular matrix (ECM) and vascular integrity (Florczak-Rzepka et al., 2012; Lakhan et al., 2013; Okazaki et al., 1979; Seo et al., 2012). MMP-9 (gelatinase B) has received considerable attention due to its involvement in a variety of vasculopathies and hemorrhagic transformation after cerebral ischemia (Asahi et al., 2001; Cui et al., 2012; Lakhan et al., 2013; McColl et al., 2010; Ramos-Fernandez et al., 2011). Elevated MMP-9 immunoreactivity has been reported to be spatially associated with microbleeds in a mouse model of CAA (Lee et al., 2003), although the irrelevance of MMP-9 with CAA-related vascular dysfunction has also been reported (Hernandez-Guillamon et al., 2012). MMP-9 is widely expressed in all vascular unit cell types such as endothelial cells, vascular smooth muscle cell, astrocytes,

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http://dx.doi.org/10.1016/j.neurobiolaging.2015.07.016

microglia, pericytes, and macrophages. Previous studies have demonstrated that Aβ induces the synthesis, release, and activation of matrix MMP-9 in cultures of these cell types (Gottschall, 1996; Guo et al., 2006; Hartz et al., 2012; Lee et al., 2003; Wang et al., 2002; Zhao et al., 2009), suggesting that Aβ may boost the effects of MMP-9 on vascular disruption. Further studies may therefore be needed to understand the pathological significance of MMP-9 in CAA and CAA-associated vascular dysfunctions.

In this report, we characterized the expression of MMP-9 in vessels of postmortem human brain tissue from CAA patients and correlated its levels with the severity of CAA. Based on these results, we established a quantitative in vivo hemorrhage assay that can visualize and quantify the magnitude of hemorrhage using multi-photon microscopy and a new tracer. We applied this assay to determine whether MMP-9 interacts with Aβ to exaggerate the vascular abnormalities and intracerebral hemorrhage (ICH) occurrence in mice. This work demonstrated that MMP-9 can trigger vascular leakage and ICH; it may act with Aβ in a synergistic way to disrupt vascular integrity, suggesting that manipulation of MMP-9 may delineate a potential therapeutic target to prevent CAA-associated hemorrhage.

2. Materials and Methods

2.1. Animals and reagents

C57/BL6 mice (male, 3–4 months old) were obtained from Charles River Laboratories. Tg2576 mice (expressing human Swedish mutation of amyloid precursor protein [APP]. Male, 12–14 months old) and their aged-matched nontransgenic littermates were bred and aged in house. All animal studies were in compliance with the Massachusetts General Hospital Animal Care and Use Committee and National Institutes of Health guidelines. MMP-9 antibody (catalytic domain) and antiglial fibrillary acidic protein (GFAP) antibody were purchased from Millipore; recombinant human MMP-9 (preactivated) was purchased from Anaspec; monoclonal antibody against human fibrin (a'-fibrin) was purchased from American Diagnostic Inc; the antifibrin antibody was labeled with DyLight 594 using a commercial kit (Thermo Scientific); Prussian blue staining kits were purchased from Polysciences, Inc.

2.2. Brain specimens

Formalin-fixed paraffin-embedded brain tissues were obtained from the occipital lobe of 10 patients diagnosed with CAA or AD combined with CAA. Sections from 5 nondemented age-matched individuals were used as controls. The neuropathologies of each case were assessed according to the ABC scoring scheme (Montine et al., 2012) (Table 1). Five of the 10 cases with moderate to severe CAA had symptomatic lobar hemorrhages (4 of the 6 CAA cases and 1 of the CAA + AD cases). All cases were from the Neuropathology Core of the Massachusetts Alzheimer Disease Research Center Brain Bank, and the study protocol was approved by the Institutional Review Board.

2.3. Severity of CAA

CAA in human tissue samples was graded with respect to the severity of the pathology as previously described with minor modifications (Greenberg and Vonsattel, 1997; Vonsattel et al., 1991). Using 0–4 scoring system, a "0" indicates no Aβ-positive blood vessels. A "1" is scattered Aβ positivity in either leptomeningeal or intracortical blood vessels. A score of "2" indicates strong, circumferential Aβ positivity in either some leptomeningeal or intracortical blood vessels. A "3" reflects full replacement of the vessel wall with amyloid plus partial or full cracking of vessel wall in leptomeningeal and intracortical blood vessels. A score of "4" is the same as 3 with additional dysphoric changes. Advanced grade 3 or 4 CAA-affected vessels demonstrate a "vessel-within-vessel" lumen, suggestive of a weakened vascular ECM resulting in the separation of intima from media during tissue preparation (Mandybur, 1986; Vonsattel et al., 1991).

Table 1
Characteristics of control and CAA subjects examined in this study

Case ID	Age	Gender	AD[a]	CAA	Group
1248	63	M	A3B3C3	Severe	AD + CAA
1023	88	M	A3B3C3	Severe	AD + CAA
1186	85	M	A3B3C3	Severe	AD + CAA
919	82	M	A3B3C3	Moderate	AD + CAA
1150	66	F	A1B2C1	Severe	CAA
821	89	M	A1B1C1	Severe	CAA
1138	75	F	A0B0C0	Severe	CAA
458	80	M	A0B1C0	Severe	CAA
753	77	F	A1B1C1	Severe	CAA
960	68	M	A1B1C1	Moderate	CAA
287	66	M	A0B0C0	Mild	Control
873	74	F	A1B1C0	Absent	Control
792	71	M	A0B0C0	Absent	Control
326	>90	M	A1B1C0	Absent	Control
373	80	F	A1B1C0	Absent	Control

Key: AD, Alzheimer's disease; CAA, cerebral amyloid angiopathy.
[a] The ABC scoring scheme (Montine et al., 2012): the A represents the assessment of amyloid burden (by the modified Thal scoring scheme); the B represents the Braak stage (consolidated from 6 tiers to 3), and the C represents the neuritic plaque score.

2.4. Immunohistochemistry

Staining was performed as previously described (Serrano-Pozo et al., 2013). Briefly, postmortem human brain sections (5 μm/section, 4 sections per case) were cleared in xylenes, rehydrated with decreasing concentrations of ethanol, and subjected to a standard antigen-retrieval procedure consisting of a 20-minute boiling in citrate buffer (0.01 M, pH 6.0) with 0.05% Tween 20. The sections were cooled at 4 °C for 30–45 minutes, blocked with 5% normal goat serum at room temperature for 1 hour, and incubated with anti-MMP-9 antibody (1:200) or anti-GFAP antibody (1:1000) overnight at 4 °C. The next day, the sections were washed 3 times (10 minutes for each wash) with Tris-buffered saline, incubated with the fluorescence-conjugated secondary antibodies, or processed with a vectastain ABC Elite kit (Vecto) and 3, 3′-diaminobenzidine (DAB) solution (DAB staining). Hematoxylin counterstaining was performed in DAB sections if needed. Negative controls were obtained by omitting the primary antibodies. Hematoxylin and eosin (H&E), thioflavin S, and Prussian blue staining were performed according to standard protocols. Images were taken with either an epifluorescence microscope (Olympus, BX61) or a confocal microscope (Zeiss, LSM510). For counting thioflavin S–positive or MMP-9-positive vessels, 4 sections from each case, 50 μm apart, were stained with thioflavin S and an anti-MMP-9 antibody. Two hundred vessels per section (800 vessels in total from each case) were randomly selected by the CAST stereology software to count the numbers of vessels with or without immunoreactivity to MMP-9 or thioflavin S. This stereology software ensures an unbiased sampling of all sections.

2.5. Craniotomy surgery

Craniotomy surgery was performed as previously described (Skoch et al., 2005). Briefly, animals were anesthetized using isoflurane (1.5%), the skin and periosteum were removed, and a

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