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Research Report

Regulation of matrix metalloproteinase 2 by oligomeric amyloid β protein☆

Wenjun Li[a], Ethan Poteet[a], Luokun Xie[a,c], Ran Liu[a], Yi Wen[a,b], Shao-Hua Yang[a,b,*]

[a]Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, Fort Worth, TX 76107, USA
[b]Institute for Aging and Alzheimer's Disease Research, University of North Texas Health Science Center, Fort Worth, TX 76107, USA
[c]Center for Autoimmune and Musculoskeletal Disease, the Feinstein Institute for Medical Research, 350 Community Drive, Manhasset, NY 11030, USA

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ABSTRACT

Matrix metalloproteinases (MMPs) are a group of proteinases that degrade components of the extracellular matrix (ECM). There is increasing evidence for a link between the activation of MMPs and Alzheimer's disease (AD) pathogenesis, in which both beneficial and detrimental actions of MMPs have been suggested. It has been demonstrated that MMPs could degrade amyloid β (Aβ) and play important roles in the extracellular Aβ catabolism and clearance. On the other hand, MMPs could contribute to AD pathogenesis by compromising the blood brain barrier and promoting neurodegeneration. In the present study, we observed that oligomeric Aβ regulates MMP2 expression in a paradoxical manner. In rat primary astrocyte cultures, oligomeric Aβ down-regulated MMP2 transcription and reduced its extracellular activity. However, in a widely used mouse model for AD, immunohistochemistry demonstrated an increase of MMP2 expression in astrocytes surrounding senile plaques in APP/PS1 transgenic mice brains. Using real-time PCR, we found that the MMP2 mRNA level was elevated in APP/PS1 transgenic mice brains. In addition, elevated mRNA levels of MMP stimulating cytokines such as IL-1β and TGFβ were found in the brains of APP/PS1 mice. Our study suggests a complex regulation of MMP2 expression by oligomeric Aβ in astrocytes. While oligomeric Aβ directly down-regulates MMP2 expression and activation in astrocytes, it induces production of proinflammatory cytokines which could serve as strong stimulators for MMP2. Therefore, the ultimate outcome of the oligomeric Aβ on MMP2 activation in astrocytes might be the combination of its direct inhibitory action on astrocyte MMP2 expression and the secondary action of inducing inflammatory cytokines.

1. Introduction

Matrix metalloproteinases (MMPs) are a large group of zinc-dependent proteinases with major function in degradation of the extracellular matrix (ECM). MMPs play critical roles in tissue remodeling in the normal physiological process. In addition, MMPs have been found to be involved in many pathological conditions such as inflammation and tumor progression

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* *Corresponding author at:* Department of Pharmacology and Neuroscience, University of North Texas Health Science Center, 3500 Camp Bowie Blvd., Fort Worth, TX 76107, USA. Fax: +1 817 735 2091.
E-mail address: shaohua.yang@unthsc.edu (S.-H. Yang).
Abbreviations: Aβ, beta amyloid; MMP, matrix metalloproteinase; AD, Alzheimer's disease; ECM, extracellular matrix

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(Birkedal-Hansen et al., 1993). MMPs are expressed in the brain and appear to be inducible under pathological conditions, with glial cells and invading inflammatory cells as their major source (Yong, 2005). On the basis of domain structure, MMPs have been classified into collagenases, gelatinases, stromelysins, and MT-MMPs, among which MMP2 (gelatinase A) and MMP9 (gelatinase B) have been most intensively studied because of their prominent role in tissue damage and repair and the ease of their identification by gelatin zymography (Rosenberg, 2009). There is increasing evidence for a link between the activation of MMPs and Alzheimer's disease (AD) pathogenesis. Interestingly, both beneficial and detrimental actions of MMPs have been suggested in AD. Deposition of improperly processed amyloid β (Aβ) peptides has been suggested to be the main causative factor for AD. In vitro studies have demonstrated that both MMP2 and MMP9 could degrade Aβ (Backstrom et al., 1996; White et al., 2006) (Yin et al., 2006). Consistently, significant increases of Aβ have been found in the brains of MMP2 or MMP9 knockout mice (Yan et al., 2006). Therefore, it is believed that both MMP2 and MMP9 play important roles in the extracellular Aβ catabolism and clearance. On the other hand, activation of MMPs could lead to damage of the blood brain barrier, hence promoting neurodegeneration in AD (Zlokovic, 2005). In transgenic mouse models of AD, a strong association between MMP activation, oxidative stress, and cerebral amyloid angiopathy has been found (Garcia-Alloza et al., 2009).

The paradoxical actions of MMPs on AD might be the consequence of neuroinflammation given the well established actions of MMPs in neuroinflammation (Mun-Bryce et al., 2002). There is mounting evidence that neuroinflammation plays active roles in both onset and progression of AD. Astrocytes are the most abundant glial cell population in the CNS and are critical for the maintenance of CNS homeostasis. Astrocytes respond to all forms of CNS insults through a process referred to as reactive astrogliosis, which has been involved in almost all neurological diseases (Sofroniew and Vinters, 2010). Along with microglia, astrocytes are directly involved in the neuroinflammatory process of AD (Heneka and O'Banion, 2007). Mouse astrocytes have been shown to be able to degrade Aβ in vitro and in situ (Wyss-Coray et al., 2003). Furthermore, MMPs have been found to contribute to Abeta catabolism (Liao and Van Nostrand, 2010; Yin et al., 2006). In primary astrocyte cultures, dissimilar actions of fresh Aβ (1–40) and Aβ (1–42) on the activation of MMP2 and MMP9 have been demonstrated (Deb et al., 2003). It has been suggested that soluble oligomeric Aβ may have more critical functions in AD compared with monomeric Aβ peptides or insoluble fibril deposits (Haass and Selkoe, 2007). In the present study, we determined the action of oligomeric Aβ on MMP2 expression and activation using primary astrocyte cultures. In addition, the expression of MMP2 was assessed in a transgenic mouse model of AD.

2. Results

2.1. *Oligomeric Aβ downregulates MMP2 expression and activation in primary astrocytes*

Synthetic amyloidogenic proteins have been widely used to study the structure, assembly, and physiological effects of both oligomeric and fibrillar forms of these proteins. However, conflicting results could arise due to the difference in the preparing of these proteins. In order to evaluate the oligomeric Aβ preparation, we analyzed the fresh Aβ and oligomeric Aβ with immunoblotting, using an Aβ specific antibody (6E10). We found that most of Aβ (1–42) exists as monomers or dimers in fresh Aβ preparations. In oligomeric Aβ preparation, high molecular weight Aβ oligomers are the predominant form. On the other hand, both fresh Aβ (1–40) and the similarly oligomeric prepared Aβ (1–40) mainly exist as monomers (Fig. 1). This is consistent with a previous report (Jarrett et al., 1993) that Aβ (1–42) has much higher tendency to form large oligomers as compared with Aβ (1–40).

We treated rat primary astrocytes with Aβ (1–42) oligomeric preparation for 24 h, and the conditioned medium was collected and evaluated for MMPs' activities by gelatin zymography. We found that the MMP2 activity in the conditioned medium was significantly decreased after 20 μM oligomeric Aβ treatment ($P<0.05$) (Fig. 2A, B). A very low level of MMP9 activity was detected in the primary astrocyte cultures (Fig. 2A, E), which is consistent with previous reports that astrocytes do not express a detectable level of MMP9 (Crocker et al., 2008; Muir et al., 2002). We further examined whether oligomeric Aβ affects MMP2 expression in astrocytes. Primary astrocytes were treated with increasing concentrations of oligomeric Aβ preparations and the cells were collected for analysis of MMP2 mRNA levels by real-time PCR. Consistent with the inhibitory action of oligomeric Aβ on MMP2 activation, expression of MMP2 was also decreased upon treatment of 20 μM oligomeric Aβ (Fig. 2C). To further determine whether the reduction of MMP2 expression and activation is due to the cytotoxic effect of oligomic Aβ, an LDH assay was conducted in the same set of cultures. No significant increase of cell death was found in astrocyte cultures upon the treatment of oligomeric Aβ (Fig. 2D). These data suggest that down-regulation of MMP2 expression in astrocytes by oligomeric Aβ is not due to the potential cytotoxic action of Aβ oligomers. Most importantly, the

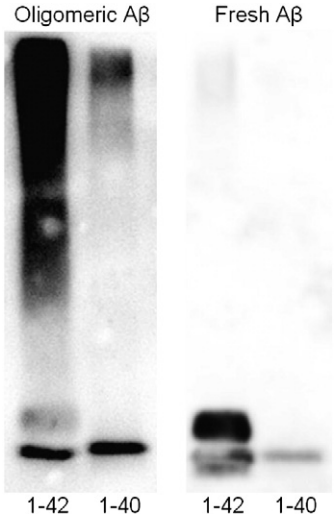

Fig. 1 – A representative Western blot of oligomeric and fresh preparations of Aβ (1–42) and Aβ (1–40). Aβ was detected with antibody 6E10.