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Brain Research

Research Report

Impacts of aging and amyloid-β deposition on plasminogen activators and plasminogen activator inhibitor-1 in the Tg2576 mouse model of Alzheimer's disease

Shin Bi Oh[a], *Nayoung Suh*[a], *Inki Kim*[a], *Joo-Yong Lee*[a,b,*]

[a]*Asan Institute for Life Sciences, Asan Medical Center, Seoul 138–736, Republic of Korea*
[b]*Department of Neurology, University of Ulsan College of Medicine, Seoul 138-736, Republic of Korea*

ARTICLE INFO

Article history:
Accepted 20 November 2014
Available online 29 November 2014

Keywords:
Amyloid pathogenesis
Amyloid plaques
Plasmin
Amyloid degrading enzymes

ABSTRACT

Plasminogen activators (PAs), which convert plasminogen into the fibrinolytic protease plasmin, may initiate the degradation of amyloid-β (Aβ) to suppress the amyloid pathogenesis. In that way, tissue plasminogen activator (tPA)-mediated plasmin activation could maintain a low level of Aβ deposition to delay the pathogenesis of Alzheimer's disease (AD). In a previous study, we reported that tPA/plasmin proteolytic activity is attenuated throughout the brain during aging or with Aβ accumulation but clustered intense around the amyloid plaques in AD brain. The present study demonstrates that the altered proteolytic activity primarily results from the competition between the expressions of tPA and plasminogen activator inhibitor-1 (PAI-1) in the brains of Tg2576 Aβ-transgenic mice, as revealed by immunohistochemistry and immunoblot assays. Compared with that in the brains of younger Tg2576 mice, tPA protein is generally reduced throughout the brain in older Tg2576 mice but elevated near amyloid plaques. In contrary, PAI-1 expression increases during aging or Aβ deposition with its clusters surrounding amyloid plaques. No significant alteration in the expression of urokinase plasminogen activator (uPA) is detected. These results suggest reciprocal feedback influences between tPA, PAI-1 and Aβ during aging and amyloid pathogenesis in AD brain; tPA-mediated plasmin activity is declined throughout the brain causing Aβ deposition during aging, and the Aβ deposits locally attract the cluster of tPA and/or PAI-1 around their deposits to competitively determine tPA/plasmin-mediated Aβ proteolysis.

*Corresponding author at: Asan Institute for Life Sciences, Asan Medical Center, Seoul 138–736, Republic of Korea.
E-mail address: jlee@amc.seoul.kr (J.-Y. Lee).

http://dx.doi.org/10.1016/j.brainres.2014.11.042
0006-8993/

1. Introduction

The main pathology of Alzheimer's disease (AD) is the massive deposition of the amyloid-β (Aβ) peptide in the brain, which is a 39–43 amino acid peptide generated from the cleavage of 680–720 amino acids of the amyloid precursor protein (APP) (Nathalie and Jean-Noel, 2008). Aβ peptides bind one another to aggregate into oligomers and fibrils, and constitute amyloid plaques.

Both tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) are serine proteases that convert plasminogen (plgn) into active plasmin. Because the fibrinolytic protease plasmin can digest Aβ peptides containing oligomers or fibrils (Exley and Korchazhkina, 2001; Ledesma et al., 2000; Van Nostrand and Porter, 1999), this plasmin proteolytic cascade modulates AD pathogenesis. In particular, tPA-activated plasmin dissolves synthetic Aβ peptides to inhibit their aggregation and attenuate their neurotoxicity (Tucker et al., 2000). A previous animal study showed that Aβ injected into the brain rapidly disappears in normal mice but persists longer to cause neuronal degeneration in tPA- or plasminogen-null mice (Melchor et al., 2003). Recently, we generated double-mutant mice overexpressing human (h)APP and deficient in tPA to reveal a markedly greater deposition of Aβ in their brains than in control mice (Oh et al., 2014).

By contrast, the results from studies examining the impact of Aβ on tPA expression or activity are conflicting. According to one point of view, Aβ, specifically its aggregate form, stimulates the transcriptional expression of tPA in cultured neurons or in the brains of hAPP-transgenic mice (Kingston et al., 1995; Tucker et al., 2000), and binds to tPA to stimulate plasmin activation (Kingston et al., 1995; Kranenburg et al., 2002). Hence, Aβ aggregates may facilitate and be degraded by the tPA/plasminogen proteolytic cascade (Melchor et al., 2003; Tucker et al., 2000). According to the other point of view, tPA expression and activity are attenuated during the deposition of Aβ in the brains of hAPP-transgenic mice (Cacquevel et al., 2007; Ledesma et al., 2000; Melchor et al., 2003).

Compared with tPA, the involvement of uPA in the pathogenesis of AD has been less well studied. Genetic analysis suggests that a genetic variant of uPA (*PLAU*) is likely associated with the pathogenesis of late onset AD (Ertekin-Taner et al., 2005; Finckh et al., 2003; Riemenschneider et al., 2006). Because the proteolytic activity of uPA is high in the euglobulin fraction of plasma in patients with AD, uPA may be a biomarker for the disease (Alonso et al., 1996). Aβ aggregates induce uPA in cultured human cerebrovascular smooth muscle cells (Davis et al., 2003) or cortical neurons as well as in the brains of hAPP-overexpressing mice (Tucker et al., 2000). The uPA-activated plasmin dissociates Aβ molecules to interfere with their accumulation and to inhibit their toxicity in cultured neurons (Tucker et al., 2002). Hence, it remains possible that uPA also co-localize with and mediate the activation of plasminogen into plasmin in zones of amyloid plaques, which in turn degrades Aβ peptides, as similar to tPA.

The expression of plasminogen activator inhibitor-1 (PAI-1) increases related to age in hAPP transgenic mice (Melchor et al., 2003; Cacquevel et al., 2007; Liu et al., 2011). A recent study further showed that PAI-1 could inhibit tPA-mediated plasmin activation leading the increase in Aβ deposition/accumulation in the brains of AD patients and mouse models (Liu et al., 2011). Therefore, it would be of interest to elaborate how the balance between PAs and PAI-1 has an influence on amyloid pathology.

In earlier studies using in situ caseinolytic zymography, we found the distributional varieties and age-related alteration of plasmin proteolytic activity in the brains of the normal and Tg2576 AD model mice (Lee et al., 2007; Oh et al., 2014). The proteolytic activity was higher in the hippocampal mossy fiber area as well as neuron cells in the normal brains, but became attenuated with age or Aβ deposition. Notably, the novel proteolytic activity developed intensified around amyloid plaques in Tg2576 mice. Although tPA is considered to be a triggering component of the Aβ–targeting plasmin proteolysis (Oh et al., 2014), the present study shows that the proteolytic activity depends on the reciprocal competition between tPA and PAI-1. We further elaborate on the influence of aging or Aβ deposition on tPA/uPA/PAI-1 expression and demonstrate their possible roles in the pathogenesis of AD.

2. Results

To examine the expression pattern of tPA in the brain, we immunologically stained tissue sections for tPA and evaluated the intensity and the distribution of the resulting immunoreactivity in the hippocampus, an area rich in tPA (Qian et al., 1993; Teesalu et al., 2004). In normal wild-type littermates, tPA immunofluorescence was most intense in the hippocampal mossy fiber area and brighter along the neuronal layers containing CA1 pyramidal and dentate granule cells (Fig. 1A and B). The tPA immunoreactivity was also found in blood vessels (arrowheads in Fig. 1B), consistent with our previous study (Lee et al., 2007). However, the intensity of immunofluorescence became weaker throughout the brain tissues at the age of 18 months (Fig. 1B and L) compared with that at the age of seven months (Fig. 1A and L), when Tg2576 mice rarely develop amyloid deposition yet despite the over-expression of hAPP (Hsiao et al., 1996). In comparison with their wild-type littermates (Fig. 1A and B), Tg2576 mice demonstrated altered patterns of tPA-immunoreactive fluorescence at the same ages (Fig. 1D and E). The tPA-immunoreactive fluorescence appeared lighter throughout the brain and was nearly absent in the granular and pyramidal neuronal layers (small arrows in Fig. 1D and E). This loss of tPA in neurons in the Tg2576 mice was evidently characterized in brain sections counterstained with a Nissl-reactive fluorochrome that specifically reacts with neuronal cell bodies (Fig. 1C, F and Fig. 2). Similar to the normal wild-type littermates, Tg2576 AD model mice also showed the diminishment of tPA-immunoreactivity in proportion to age, as compared the brain tissues between 7-month- and 18-months-old mice (Fig. 1D, E, and L). By contrast, tPA and plasminogen immunofluorescence was intensified at the periphery of the compact core of amyloid plaques and the vessel walls, which were immunoreactive to Aβ42 (Fig. 2), repeating our previous findings (Lee et al., 2007). The tPA immunoreactivity was also intense in the reactive glial cells adjacent to the amyloid plaques (arrowheads in Fig. 2 A–C). Notably, except for