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ALZHEIMER'S THERAPEUTICS: Anti-Amyloid

The Possible Role of Tissue-Type Plasminogen Activator (tPA) and tPA Blockers in the Pathogenesis and Treatment of Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is the leading cause of cognitive decline in aged individuals. The pathological hallmarks of AD include the formation of neurofibrillary tangles, along with senile plaques that are mainly composed of the amyloid-β (Aβ) peptide. Several lines of evidence implicate the tPA/plasmin system in AD. One type of cell death observed in AD is excitotoxic neuronal damage, and the tPA/plasmin system participates in excitotoxic cell death. Recent in vitro experiments report that the addition of aggregated Aβ peptide to primary cortical neurons leads to the up-regulation of tPA mRNA expression. Additionally, plasmin (activated by tPA) attenuates Aβ neurotoxicity by degrading the peptide and rendering it inactive. However, there is no evidence to demonstrate an in vivo contribution of the tPA/plasmin system in AD. We are currently examining the effects of the tPA/plasmin system on the deposition and toxicity of the Aβ peptide with in vivo paradigms of AD. We hope to define the contribution of the tPA/plasmin system in the development of AD pathology.

Index Entries: tPA; plasmin; Aβ deposition; Aβ degradation.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia and intellectual failure in aged individuals. People with AD have several characteristic pathologies, including the formation of senile plaques composed of the amyloid-β (Aβ) protein, the accumulation of neurofibrillary tangles, and the deposition of Aβ within the small- to mid-sized blood vessels within the brain, a condition called cerebral amyloid angiopathy (CAA) (for recent review, *see* Selkoe, 2001). Some forms of early-onset familial AD can be traced to mutations within three genes encoding the Aβ protein precursor (AβPP), presenilin-1, and presenilin-2 (PS-1, PS-2) (Tanzi et al., 1996; Selkoe, 2001). These mutations lead to an increase in the production of the Aβ protein from its parent molecule AβPP (Citron et al., 1992; Haas et al., 1992; Duff et al., 1996). This increased Aβ production and its subsequent deposition in the parenchyma has been thought to be a key development in AD.

Plasminogen activators (PAs) are serine proteases whose main function is to activate plasminogen into plasmin, a protease capable of degrading a variety of proteins (for review, *see* Vassali et al., 1991). There are two types of PAs in mammals: tissue-type (tPA) and urokinase-type (uPA). tPA is expressed in various regions of the mouse brain, including the hippocampus, amygdala, cerebellum, and hypothalamus, where there is evidence that tPA is responsible for normal as well as pathological functions (Sappino et al., 1993). In particular, tPA is

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expressed highly in the mouse hippocampus, a region important for learning and the forming of memories.

Studies in our laboratory have provided a link between tPA and neuronal degeneration (Tsirka et al., 1995, 1996, 1997; Chen and Strickland, 1997). Using an excitotoxin-induced mouse model for neuronal death (kainate injection), tPA and plasmin were linked with damage in the hippocampus. Plasmin activation leads to the cleavage of the extracellular matrix protein laminin sensitizing the neurons to excitotoxic stress (Chen and Strickland, 1997).

Numerous reports have linked the tPA/plasmin system to AD. It was shown that aggregated Aβ stimulated tPA activity (Kingston et al, 1995). In neuronal cell cultures, tPA activation of plasminogen to plasmin leads to Aβ degradation and the attenuation of the peptide's toxicity (Tucker et al., 2000a, 2000b). Although this suggests a beneficial effect of tPA against AD development, there has not yet been a definitive study on the role that tPA plays on the progression of this disorder. It is also not known whether the increase of tPA activity reflects a compensatory mechanism to attenuate the onset of AD or whether tPA triggers a cell death similar to that observed in kainate-induced hyperexcitation. We plan to define the function (and therapeutic potential) of molecules in the tPA/plasmin system on the deposition of Aβ in mouse models of AD.

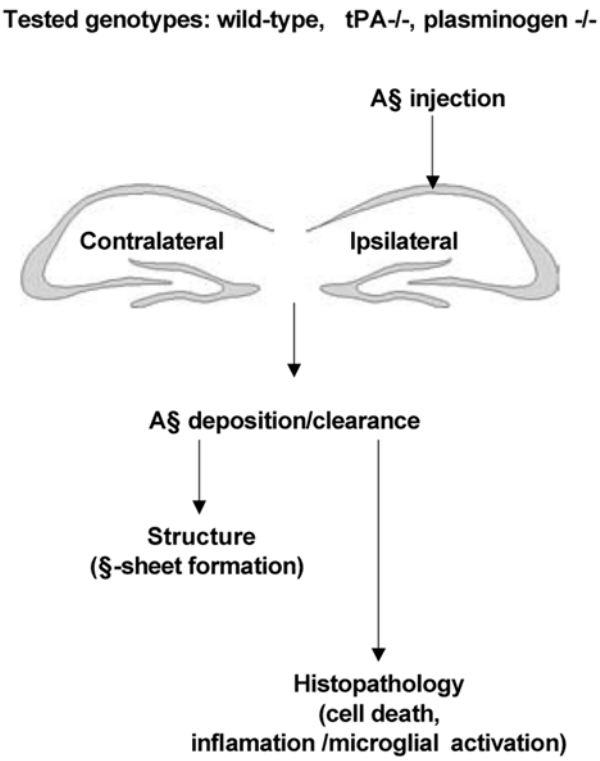

Fig. 1. Model to assess the role of tPA/plasmin system in the development of AD pathology. Mice deficient in either tPA or plasminogen are injected with the Aβ peptide into the CA1 region of the hippocampus. The deposition and clearance of Aβ are observed along with any changes in Aβ structure. Ultimately, the pathology caused by Aβ deposition is noted.

Materials and Methods

Mice are injected with $A\beta_{40}$ peptide freshly solubilized to 2 μg/μL in phosphate-buffered saline. For injection into the hippocampus, the coordinates relative to bregma (anterior/posterior, 2.5 mm; dorsal/ventral, 1.7 and 1.8 mm in depth) are taken from *The Mouse Brain in Stereotaxic Coordinates* (Franklin and Paxinos, 1997) and measured using a Kopf Company stereotaxic instrument. Animals are allowed to recover for 3 d prior to isolation of the brains.

Thioflavin S (Th S) staining is done according to Wyss-Coray et al. (1997). Briefly, every sixth coronal section is incubated in 1% Th S for 8 min, followed by rapid rinses in 100% EtOH, 80% EtOH (2×), and water (10 min). The sections are viewed under a fluorescence microscope.

For Aβ immunostaining, sections are incubated with a rabbit polycolonal anti-Aβ antibody (1 : 1000) overnight (Biosource). Sections are then incubated with secondary antibody (1 : 200), followed by an ABC kit (Vector Labs). Aβ immunoreactivity is visualized using Nova-Red (Vector Labs).

Cresyl violet and Fluoro-Jade B stainings are used to assess neurodegeneration. Briefly, cresyl violet working solution is prepared by adding 20 mL of 0.1% cresyl violet acetate to 90 mL Walpole buffer. The sections are incubated in vresyl violet solution for 15 min at room temperature, followed by rinses with 95% EtOH, 100% BuOH for 2 min (2×), and Histoclear (2×) (National Diagnostics). The slides are viewed under light microscopy. Fluoro-Jade B staining is done according to the protocol by Schmued and Hopkins (2000) and viewed under a fluorescence microscope.

The experimental paradigm is shown in Fig. 1.

Discussion

The presence of Aβ senile plaques is a characteristic pathology of AD. This Aβ deposition may be caused by the increased level of the Aβ, possibly because of an imbalance in the production and clearance of peptide. Recent reports have identified sev-

eral enzymes (insulin-degrading enzyme, endothelin-converting enzyme, neprilysin, plasmin) capable of degrading Aβ (Vekrellis et al., 2000; Tucker et al., 2000a; Eckman et al., 2001; Iwata et al., 2001). Another group has reported differing actions of protease inhibitors, when coinfused with Aβ, on the deposition and toxic effects of the peptide (Frautschy et al., 1998). Additionally, initial results from our laboratory indicate that the tPA/plasmin system might be important in the deposition of Aβ in the mouse hippocampus.

To help answer this and other questions about tPA/plasmin system involvement in AD, we plan to use both biochemical and genetically based experiments to resolve the mechanism for the effects of tPA on AD progression. Furthermore, the probablility of molecules within the tPA/plasmin system emerging as therapeutic agents for AD can also be examined.

The potential role of the tPA/plasmin system in AD is not yet clearly defined. We are currently looking at several different methods to identify this system's possible contribution to the pathogenesis of AD. By describing the function of the tPA/plasmin system in the development of AD, it might be possible to develop drugs based on tPA/plasmin molecules to help alleviate the symptoms and pathology seen in AD.

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References

Chen Z.-L. and Strickland S. (1997) Neuronal death in the hippocampus is promoted by plasmin-catalyzed degradation of laminin. *Cell* **91,** 917–925.

Citron M., Oltersdorf T., Haass C., McConlogue L., Hung A. Y., Seubert P., et al. (1992) Mutation of the β-amyloid precursor protein in familial Alzheimer's disease increases β-protein production. *Nature* **360,** 672–674.

Duff K., Eckman C., Zehr C., Yu X., Prada C. M., Perez-tur J., et al. (1996) Increased amyloid-β 42(43) in brains of mice expressing mutant presenilin 1. *Nature* **383,** 710–713.

Eckman E. A., Reed D. K., and Eckman C. B. (2001) Degradation of the Alzheimer's amyloid β peptide by endothelin-converting enzyme. *J. Biol. Chem.* **276,** 24,540–24,548.

Franklin K. B. J. and Paxinos G. (1997) *The Mouse Brain in Stereotaxic Coordinates.* Academic Press, Inc., San Diego, CA.

Frautschy S. A., Horn D. L., Sigel J. J., Harris-White M. E., Mendoza J. J., Yang F., et al. (1998) Protease inhibitor coinfusion with amyloid beta-protein results in enhanced deposition and toxicity in rat brain. *J. Neurosci.* **18,** 8311–8321.

Haass C., Schlossmacher M. G., Hung A. Y., Vigo-Pelfrey C., Mellon A., Ostaszewski B. L., et al. (1992) Amyloid β-peptide is produced by cultured cells during normal metabolism. *Nature* **359,** 322–325.

Iwata N., Tsubuki S., Takaki Y., Shirotani K., Lu B., Gerard N. P., et al. Metabolic regulation of brain Aβ by neprilysin. *Science* **292,** 1550–1552.

Kingston I. B., Castro M. J., and Anderson S. (1995) In vitro stimulation of tissue-type plasminogen activator by Alzheimer Amyloid beta-peptide analogues. *Nat. Med.* **1,** 138–142.

Sappino A.-P., Madani R., Huarte J., Belin D., Kiss J. Z., Wohlwend A., and Vassali J.-D. (1993) Extracellular Proteolysis in the adult murine brain. *J. Clin. Invest.* **92,** 679–685.

Schmued L. C. and Hopkins K. J. (2000) Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res.* **874,** 123–130.

Selkoe D. J. (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.* **81,** 742–760.

Tanzi R. E., Kovacs D. M., Kim T.-W., Moir R. D., Guennette S. Y., and Wasco W. (1996) The gene defects responsible for familial Alzheimer's disease. *Neurobiol. Dis.* **3,** 159–168.

Tsirka S. E., Gualandris A., Amaral D. G., and Strickland S. (1995) Excitotoxin-induced neuronal degeneration and seizure are mediated by tissue plasminogen activator. *Nature* **377,** 340–344.

Tsirka S. E., Rogove A. D., and Strickland S. (1996) Neuronal cell death and tPA. *Nature* **384,** 123–124.

Tsirka S. E., Rogove A. D., Bugge T. H., Degen J. L., and Strickland S. (1997) An extracellular proteolytic cascade promotes neuronal degeneration in the mouse hippocampus. *J. Neurosci.* **17,** 543–552.

Tucker H. M., Kihiko M., Caldwell J. N., Wright S., Kawarabayashi T., Price D., et al. (2000a) The plasmin system is induced by and degrades amyloid-beta aggregates. *J. Neurosci.* **20,** 3937–3946.

Tucker H. M., Kihiko-Ehmann M., Wright S., Rydel R. E., and Estus S. (2000b) Tissue plasminogen activator requires plasminogen to modulate amyloid-beta neurotoxicity and deposition. *J. Neurochem.* **75,** 2172–2177.

Vassalli J.-D., Sappino A.-P., and Belin D. (1991) The plasminogen activator/plasmin system. *J. Clin. Invest.* **88,** 1067–1072.

Vekrellis K., Ye Z., Qiu W. Q., Walsh D., Hartley D., Chesneau V., et al. (2000) Neurons regulate extracellular levels of amyloid beta-protein via proteolysis by insulin-degrading enzyme. *J. Neurosci.* **20,** 1657–1665.

Wyss-Coray T., Masliah E., Mallory M., McConlogue L., Johnson-Wood K., Lin C., and Mucke L. (1997) Amyloidogenic role of the cytokine TGF-β1 in transgenic mice and Alzheimer's disease. *Nature* **389,** 603–606.