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RESEARCH HIGHLIGHT

Evoking plasmin for β-amyloid clearance

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common cause of dementia. It is estimated that there are currently about 18 million people worldwide suffering from Alzheimer's disease, and this number will double by 2 025 to 34 millions (http://www.alz.org). Enormous efforts have been taken to identify key molecular events underlying AD pathogenesis and seek for measures to postpone the onset and progression of, if not prevent the disease.

In the first case report of AD in 1907, Alois Alzheimer characterized the two major pathologic and diagnostic hallmarks of the disease. One is the neurofibrillary tangles, composed mainly of hyperphosphorylated microtubule-associated protein tau accumulated as paired helical filaments (PHFs). Another is the senile plaques, composed of fibrillar and amorphous Aβ (amyloid-beta peptide) aggregates [1]. Abnormal hyperphosphorylation of tau is proposed to destroy microtubule dynamics and disrupt neuronal transportation, whereas Aβ exerts a range of cytotoxic actions and directly impairs brain cognitive function [2-4]. Accumulating genetic and biochemical evidence supports the notion that Aβ is the central feature in the pathogenesis of AD.

The Aβ peptide is generated by endoproteolysis of the ubiquitously expressed single-transmembrane β-amyloid precursor protein (APP). APP is processed in two different ways: a benign pathway, in which α-secretase cleaves the Aβ domain in APP, thereby precluding formation of intact Aβ; and an amyloidogenic pathway, in which Aβ is released from APP by β-cleavage at the N terminus followed by γ-cleavage at the C terminus. The aspartyl proteases tumor necrosis factor α converting enzyme (TACE) or a distintegrin and metalloproteinase 10 (ADAM10), also referred to as α-secretase, mediate α-cleavage of APP within its lumenal/extracellular domain [5]. Alternatively, cell surface APP is internalized into early endosomes, where it is cleaved at a more distal site along the lumenal/extracellular domain by β-site-APP-cleaving enzyme (BACE, β-secretase), and then by an intramembrane γ-secretase cleavage that yields predominantly a 40-mer peptide (Aβ40) and smaller amounts of a 42-mer peptide (Aβ42) [6]. As reduction of Aβ production should attenuate the formation of amyloid plaque and alleviate neurodegeneration, β-secretase and γ-secretase become the key therapeutic targets for AD. On the other hand, enhancement of α-cleavage would reduce the generation of Aβ. Thus augmentation of α-secretase activity is also encouraging for therapeutical exploration. However, in addition to APP, these enzymes all have several other substrates that are involved in cellular procedures, which makes the pursuit of specific and effective inhibitors/activators challenging.

The steady-state level of Aβ peptide is determined not only by Aβ production, but also by Aβ clearance. As abnormal activation of β- and γ-secretase links to Aβ accumulation, under-activated Aβ-degrading enzymes contribute to anomalous Aβ deposition in the brain as well. So far, several proteases have been shown to be responsible for Aβ removal. Evidence from animal studies strongly suggests that neprilysin (NEP) [7] and insulin-degrading enzyme (IDE) [8] are the key Aβ degradation enzymes *in vivo*. Besides NEP and IDE, the endothelin-converting enzymes (ECE1 and ECE2), angiotensin-converting enzyme (ACE), matrix metalloproteinase-9 (MMP-9) and plasmin are shown to participate in Aβ degradation *in vitro*. However, their physiological roles in Aβ clearance *in vivo* is still inconclusive [9]. All these Aβ-degrading enzymes have distinct subcellular localizations, and are mediated by different cellular signals. Upregulation of these enzyme expression levels or their proteolytic activities, especially by certain pharmaceutical agents, may provide novel and viable therapeutic strategies for AD. In a recent issue of *PNAS*, Jacobsen *et al.* [10] from Wyeth Research show that enhancement of the plasmin cascade by a small compound PAZ-417 increases Aβ catabolism *in vitro* and ameliorates amyloid-related pathology.

The plasmin cascade is responsible for the fibrinolysis in blood. In brain, the plasmin cascade is important for maintenance of extracellular matrix dynamics during neuronal development and for events that require synaptic plasticity such as long-term potentia-

tion, memory and learning [11]. Active tPA (tissue-type plasminogen activator) and uPA (urokinase-type plasminogen activator) cleave the proenzyme plasminogen to generate the active serine protease, plasmin. Plasminogen activators (PAs) are in turn controlled by plasminogen activator inhibitors (PAI-1 and PAI-2). Recent studies by several groups have shown that Aβ aggregates can substitute fibrin aggregates to stimulate expression of tPA and uPA and be degraded by the plasmin system. Of additional interest is that plasmin has been shown to not only digest released Aβ peptide but also be active for APP α-cleavage. However, mice deficient in plasminogen do not have elevated Aβ level, suggesting that plasmin might not regulate the steady-state levels of Aβ under non-pathogenic conditions but may be involved in Aβ clearance after aggregation is initiated. Furthermore, in addition to a correlation of increased plasmin activity with bleeding problem, enhancement of plasmin cascade has been found in neuronal death in certain pathologies such as seizure and stroke. Increased tPA synthesis and plasmin generation leads to laminin degradation and contributes to neurotoxicity in ischemia. Thus the side effects linked with direct activation of PAs and/or plasmin have to be carefully evaluated. The study by Jacobsen and his colleagues presents us with an alternative. They chose PAIs as the drug target for upregulation of the plasmin cascade. These authors characterized PAZ-417 in a systematic screening for biologically active compounds that regulate PAI-1 activity, and demonstrated this inhibitor could accelerate the degradation of Aβ and consequently ameliorate β-amyloid pathology *in vivo*.

This work builds on their observation that the expression levels of hippocampal tPA are comparable but the activities are strikingly declined in AD mice compared to that of control mice. On the other hand, PAI-1 is markedly elevated in AD mouse hippocampus. The authors show that inhibition of PAI-1 partially rescued hippocampal tPA activities. By using a reconstituted assay system, Jacobsen *et al.* demonstrate that tPA/plasminogen mediated Aβ degradation is abolished by PAI-1 *in vitro*. The inhibition of plasmin-mediated Aβ clearance by PAI-1 can be dose-dependently eliminated by the specific inhibitor PAZ-417. Then, would treatment with PAI-1 inhibitors enhance Aβ catabolism *in vivo*? Results from *ex vivo* and *in vivo* assays clearly indicate that activation of plasmin by PAZ-417 treatment lowers Aβ levels, and thus reverses hippocampal LTP and memory deficits in AD transgenic mice. Interestingly, though the authors did observe decrease of plasma Aβ as well as brain Aβ levels, only the brain-penetrating PAI inhibitor PAZ-417 improves memory deficiency, suggesting aberrant inactivation of central tPA by PAI-1 but not that of peripheral PAI-1 contributes to AD pathology. In this context, further evaluation of the status of local plasmin cascade in brain and appraisement of strategies for its manipulation should be of great therapeutic interests.

At present, the only available treatments for AD mainly act symptomatically and improve cognitive functions temporarily. Compounds targeting secretases or Aβ vaccination have been obstructed in therapeutic trials. Upregulation of the amyloid-degrading enzymes provides alternative options. While gene therapy approaches might be viable to selectively increase the enzyme levels, a more practical approach would be to augment their activity pharmacologically. Furthermore, pharmacological means manipulating the cellular pathways that regulate the activities of these enzymes would be capable of enhancing the spatial specificity of the enzymes and efficacy for Aβ clearance. In their report, Jacobsen and colleagues have provided a proof of concept. Further exploration of diverse potential regulatory mechanisms of Aβ metabolism should pave the way for developing new therapeutic strategies for AD.

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