Editorial

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Future Medicinal Chemistry

Can small molecule inhibitors of glutaminyl cyclase be used as a therapeutic for Alzheimer's disease?

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Alzheimer's disease (AD) is a multifactorial and socioeconomically burdensome disease. In view of the failures of anti-AD candidates, we should try to rethink what we did before and what we should do next, in part at least. Research shows that the more neurotoxic factor, pyroglutamate-Aβs, and the more important inflammatory mediators, pyroglutamate-CCL2, both contribute to the initiation of AD specifically and the generation of N-terminal intramolecular cyclization catalyzed by glutaminyl cyclase quality control, the over-expression of which correlates positively with the severity of AD. Subsequently, lowering pyroglutamate-Aβs and pyroglutamate-CCL2 levels by quality control inhibition using small molecule inhibitors could be expected as an amazing strategy for the prevention and treatment of AD.

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Alzheimer's disease (AD) is a systematically chronic neurodegenerative disease characterized by diminution of cognitive function, memory, neurobehavioral manifestations, social withdrawal and other behavioral symptoms. AD is the most common cause of dementia in aging and accounts for 60–80% of all cases. The prevalence of AD has increased dramatically over the past decade and is expected to become even worse in the future owing to increased life expectancy. Unfortunately, we have almost no idea about the exact pathology of this multifactorial disease, and there is no cure, at least no disease modifying agents, for this socioeconomically burdensome disease. For these reasons, demand for development of appropriate prevention and/or treatment options for AD will continue to grow rapidly.

As one of the hallmarks, extracellular amyloid plaques contribute to the death of neurons and development of AD. β-Amyloid (Aβ) pathology is well documented, and the Aβ cascade hypothesis has been one of the main hypotheses. However, the fact is no candidate targeting Aβ pathology directly has passed through clinical trials including the failure of Lilly's solanezumab and Merck's verubecestat. Obviously, the development of anti-AD agents is a world challenge now. We cannot judge that the amyloid cascade hypothesis is flawed even now, but is Aβ aggregate the reasonable target for the discovery of anti-AD agents? Do we have any 'misunderstanding' about the AD pathology? What is the problem with the development strategy we use? Maybe it is the time for us to change our chair and rethink in part at least what we saw, what we did, what we learned and what we should do next.

Many studies confirm that the formation of Aβ plaques, mainly composed of $Aβ_{42}$ and $Aβ_{40}$, has no direct effects on the development of neurodegeneration and other clinical AD symptoms. These plaques can be detected in both AD brains and normal brains. What is the pathological difference between these two types of brain? In recent years, a variety of N-truncated Aβs have been identified only in AD brains including pyroglutamate-Aβs (pE-Aβs), which are the generations of Aβs undergoing N-terminal truncation by two or ten amino acids and following cyclization of resulting N-terminal glutamate (E) to pyroglutamate (pE). These pE-Aβs, especially pE-$Aβ_{3–40/42}$, account for more than 50% of the total plaques in AD brains [1]. Compared with Aβs, pE-Aβs confer proteolytic resistance, increased hydrophobicity, aggregate more rapidly and seed further Aβ aggregation. Meanwhile, pE-Aβ can drive the downstream toxicity cascade to destroy the plasticity of synapses and induce the death of neurons through the formation of much stronger neurotoxic oligomers and 'infection' of Tau, ROS, Ca^{2+} and other pathways [2–4].

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These findings suggest that pE-Aβs may be one of the real causes of AD, especially in the early stage before the Aβ aggregates could be detected. Inhibiting the generation of pE-Aβs is a good option for the development of anti-AD agents because of pE-Aβs' crucial role.

Inflammation is another important risk component in the initiation of AD. Although, we do not know the exact mechanism yet [5]. Neuroinflammation is clinically characterized by activated microglia and astrocytes surrounding the plaques in the brain. Among the inflammatory mediators, CCL2, also known as MCP-1 mainly recruits monocytes and macrophages and exhibits its biological activity through the binding with CCR2. CCL2 attributes to the activation of microglia and astrocytes, in turn, activated microglia and astrocytes can secrete more CCL2 and other inflammatory molecules. Decreasing CCL2 level may contribute to treatment of inflammatory diseases, including AD [6,7]. However, N-terminal modified CCL2, pE-CCL2, may be the more important inflammatory component in the course of AD compared with CCL2. This cyclized form of CCL2 is also generated by N-terminal cyclization of resulting E to pE, which confers resistance against degradation by aminopeptidase and is more active in chemotaxis assays. As a result, pE-CCL2 exhibits much more functional CCL2 activity *in vivo* [8]. The generation of pE-CCL2 is initiated in AD patients decades before the formation of Aβ fibrils. And the pE-CCL2 levels in cerebrospinal fluid correlates with the faster cognitive decline in prodromal AD patients. Accordingly, inhibiting the generation of pE-CCL2 is believed to be a promising new approach for the prevention and treatment of neuroinflammation at the beginning of AD [9].

Considering the specific presence of pE-Aβs and pE-CCL2 and the key role during the initiation and development of AD, lowering pE-Aβs and pE-CCL2 levels by inhibiting their generation, could be expected as an amazing strategy for the discovery of novel anti-AD chemicals, and method for the prevention and treatment of AD. pE-Aβs and pE-CCL2 are both the generations of N-terminal intramolecular cyclization. Normally, this cyclization of peptide/protein N-terminal glutamine residues into pE is important and a common post-translational event for maturation of bioactive neuropeptides, hormones and cytokines in the secretory pathway. This cyclization was thought to be a spontaneous reaction, however, it has now been approved that this conversion is mainly catalyzed by glutaminyl cyclase (QC, also known as QPCT, EC 2.5.2.3), which is widely distributed in mammalian brain with robust expression in the hippocampus and cortex. The higher expression of QC is directly associated with several complex pathologies ranging from inflammation to neurodegenerative diseases. The generation of pE-Aβs and pE-CCL2 are both catalyzed by the over expressed QC, and QC expression level correlates positively with the accumulation of pE-Aβs and pE-CCL2 in AD brains and the severity of AD [8,10]. Furthermore, compared with age-matched normal brains, much higher QC mRNA levels are found in AD, and the higher expression of QC can even be seen in peripheral blood before any other AD related biomarkers can be detected [11]. According to this research, QC inhibition may offer a new opportunity for the prevention and treatment of AD by inhibiting the generation of pE-Aβs and pE-CCL2 and blocking the related pathway consequently in the earliest stage.

To inhibit the activity of QC, a few small molecule QC inhibitors have been reported [12–16]. These chemicals contain an aromatic motif tethered to an imidazole moiety, where the aromatic ring matches the hydrophobic space at the entrance of the active site and the imidazole moiety binds the catalytic zinc ion at the bottom of the pocket. These inhibitors were found to be efficient in reducing the generation of pE-Aβs and pE-CCL2 *in vitro* and *in vivo.* Further assessments corroborated that these compounds can reduce monocyte/macrophage infiltration, exhibit strong anti-inflammatory actions in many inflammation models, and improve the behavior and reduce AD pathology in several transgenic mouse models. Meanwhile, it is inspiring to see that PQ912, a competitive inhibitor of QC, inhibits QC activity in cerebrospinal fluid by 92%, exhibits a benefit on working memory and a trend on attention in Phase II SAPHIR trial. The synaptic marker neurogranin and the inflammatory marker YKL40 trend downward after the treatment of PQ912 in these enrolled people with mild cognitive impairment or mild dementia [17]. So, small molecule inhibitors of QC may represent an alternative therapeutic strategy to treat AD.

As we know, blockade of pE formation by inhibiting QC may have unpredictable side effects because pE is also the N-terminal of many other proteins, hormones and peptides. With this potential issue in mind, discovery of inhibitors with a novel skeleton and further understanding of QC biology will hopefully lead to the development of disease-modifying agents for the treatment of AD.

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