รายงานวิจัยฉบับสมบูรณ์

ปฏิกิริยาสัมพันธ์ระหว่าง chimeric TTR กับโปรตีนใน CSF และผลกระทบต่อความสามารถของ chimeric TTR ในการยับยั้งและสลาย amyloid β

Interaction between chimeric TTR and protein(s) in CSF, and its effect on abilities of chimeric TTR to inhibit and disrupt amyloid β

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TABLE OF CONTENT

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ABSTRACT

Alzheimer's disease (AD), one of the most frequent types of amyloidosis, is commonly associates with dementia in human. The extracellular deposition as amyloid plaque and toxicity to cells of amyloid β (Aβ) are common causes. Thus, removal of Aβ is the most important therapeutic strategy for AD. Besides function as a distributor for thyroid hormones (THs) and retinol, transthyretin (TTR) contains a proteolytic activity and it is known as a major Aβ sequestering protein in human brain. Recently, chimeric TTRs with higher proteolytic activity than wild type TTR were constructed in our laboratory. To ensure these TTRs can be used as potent therapeutic agents, we investigated their proteolytic activities both *in vitro* and *in vivo*. In addition, to explore a possible effect of particular proteins in the CSF such as metallothinein (MT) on the degradation of Aβ, the experiment was also carried with the presence of the CSF protein. The recombinant chimeric TTRs including xenoN/crocTTR and pigC/crocTTR were successfully synthesized and secreted by using the heterologous gene expression system of *Pichia pastoris*. The purification by preparative discontinuous native-PAGE showed only a single band with a migration to the position corresponding to TTR. The synthesized TTRs contain the physicochemical characteristics similar to those observed in the native TTRs. By using FITC-casein as substrate, the results showed that crocTTR with longer N- or C-terminal sequence had higher catalytic activity than wild type crocTTR, indicating to the effect of N- and C-terminal sequences on the activity of TTR. In addition, neither the presence of metallothionein 1 nor metallothionein 2 (MT1 and MT2) changed the $A\beta_{1-42}$ degradation activity of human TTR. On the other hand, the degradation of $A\beta_{1-42}$ by the two chimeric TTRs was enhanced with the presence of either MT1 or MT2. We also determined whether the two chimeric TTRs toxic to cells and/or have the protective effect on the toxicity of Aβ. The cytotoxicity assay was conducted using neuroblastoma as cell target and the cell viability was determined by MTT assay. The results showed that the viability of cells with the presence of TTR was significantly higher than the control in which cell was treated with Aβ alone. In comparison with human TTR, crocTTR and chimeric TTR (either xenoN/crocTTR or pigC/crocTTR) showed more protective effect.

However, there was no significantly difference of the effect between xenoN/crocTTR and pigC/crocTTR. This indicated to non-toxic but ability to protect cell of the chimeric TTRs.

INTRODUCTION

Alzheimer's disease (AD) is one of the most frequent types of amyloidosis which commonly associates with dementia in human. The extracellular deposition of amyloid β (Aβ) in the form of amyloid plaque is a common characteristic. The ineffective Aβ clearance processes by enzyme-mediated degradation (Saido and Iwata, 2006; Miners et al., 2008; Leissring, 2008) in particular has been strongly suggested leading to the abnormal accumulation of the peptide (Mawuenyega et al., 2010). Although the deposition of Aβ aggregates has been known to be a critical step of the disease, the mechanism by which Aβ forms aggregates is still unclear. The steady-state level of Aβ in the normal brain is maintained by the balance between its production and clearance. Failure of this balance either over-production of Aβ or a reduction in Aβ can lead to development of AD. Therefore, removal of Aβ from the brain is the most important therapeutic strategy for AD. In nature, few extracellular proteins in the CSF can sequester Aβ, and several enzymes have been identified for their capabilities of degrading Aβ in the brain and plasma. Among these proteins, transthyretin (TTR) is known as a major Aβ sequestering protein in human brain (Schwarzman et al., 1994). It has been shown that the binding of TTR to soluble Aβ prevents the fibrillation of the peptide (Golabek et al., 1995). In addition, the inhibition/disruption of Aβ fibrils is hypothesized as a possible mechanism underlying the protective role of TTR in AD (Costa et al., 2008). The cleavage of Aβ by TTR was recently explored, and the Aβ fragments that generated from the cleavage showed lower amyloidogenic potent (for review, see Liz et al., 2010).

In human, there are two major sites of TTR gene expression i.e. the liver and epithelial cells of choroid plexus of the brain (Soprano et al., 1985), which secretes the protein into plasma and CSF, respectively. The primary structure of TTR subunit is highly conserved during the evolution of vertebrates. The amino acid sequence alignment of TTR subunits from vertebrate species shows that all amino acid residues in the central channel, including those involved in the binding interaction with THs, are conserved and have not been altered for more than 400 million years (Blake and Oatley, 1977; Wojtczak et al., 1996; Blake, 1981). The only predominant change in amino acid residues occurs at N- and C- terminal