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# Acetyl-Ile-Gly-Leu protects neurons from $A\beta_{1-42}$ induced toxicity in vitro and in V337M human tau-expressing mice

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ABSTRACT

*Aims:* We previously reported that the neurotoxicity of amyloid β protein ($A\beta_{1-42}$, 10 nM) was blocked by an Aβ-derived tripeptide, $A\beta_{32-34}$ (Ile-Gly-Leu, IGL), suggesting that IGL may be a lead compound in the design of Aβ antagonists. In the present study, three stable forms of IGL peptide with acetylation of its N-terminal and/or amidation of its C-terminal (acetyl-IGL, IGL-$NH_2$ and acetyl-IGL-$NH_2$) were synthesized and examined for their effects on Aβ-induced neurotoxicity.
*Main methods:* Phosphatidylinositol 4-kinase type II (PI4KII) activity was measured using recombinant human PI4KIIα kinase and cell viability was assessed in primary cultured hippocampal neurons. To test effects in vivo, 1.5 μl of 100 nM Aβ and/or 100 nM acetyl-IGL was injected into the hippocampal CA1 region of right hemisphere in transgenic mice expressing V337M human tau protein. Four weeks later, behavior performance in the Morris water maze was tested and after another 2 weeks, sections of brain were prepared for immunohistochemistry.
*Key findings:* Among the three modified tripeptides, acetyl-IGL attenuated the Aβ-induced inhibition of PI4KII activity as well as enhancement of glutamate neurotoxicity in primary cultured rat hippocampal neurons. Injection of Aβ into the hippocampus of mice impaired spatial memory and increased the number of degenerating neurons in bilateral hippocampal regions. Co-injection of acetyl-IGL prevented the learning impairment as well as the neuronal degeneration induced by Aβ.
*Significance:* These results suggest that a modified tripeptide, acetyl-IGL, may be effective in the treatment of Alzheimer's disease.



## Introduction

The amyloid β proteins (Aβs) cascade hypothesis, which focuses on the deposition and the toxicity of fibrillar Aβs, has been widely accepted as one of important mechanisms in the pathogenesis of Alzheimer's disease (AD) (Selkoe, 2001). Recently, however, this traditional hypothesis is being revised to a synaptic Aβ hypothesis which implies that non-fibrillar Aβ oligomers play a crucial role in disrupting synaptic function, which correlates very well with cognitive decline in AD patients (Tanzi, 2005). This new understanding in amyloid hypothesis is particularly interesting in light of further studies on the toxicities of other forms of Aβ, e.g. the monomer, especially in the early stages of AD when the concentration of Aβ is fairly low but probably triggers some pathological processes. Although pathophysiological concentrations of Aβs reportedly affect phospholipase $A_2$ activation at 0.24 nM (Lehtonen et al., 1996); block nicotine-induced TrkA expression and reduce cell viability in PC12 cells at 10–100 nM (Li et al., 2005); suppress spontaneous synaptic activity at 8 nM (Nimmrich et al., 2008); and modulate glutamate-mediated neurotransmission in rat basal forebrain at 100 nM (Chin et al., 2007), the direct targets of Aβ at such low concentrations are not known. Putative targets of low molecular weight Aβ at low concentrations and novel preventive or therapeutic strategies for AD through these targets need to be elucidated.

We previously demonstrated that phosphatidylinositol 4-kinase type II (PI4KII) is a target of pathophysiological concentrations of Aβs (≤10 nM) (Wu et al., 2002, 2004, 2006). Most recently, a short peptide, Ile-Gly-Leu (IGL), corresponding to the amino acid sequence of $A\beta_{32-34}$ was found to antagonize the neurotoxicity of 10 nM Aβ via attenuation of the Aβ-induced inhibition of PI4KII activity, indicating the IGL sequence to be a potential lead compound in the design of a novel Aβ antagonist for the treatment of AD (Xiong et al., 2007). In this study, to

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minimize the breakdown of the IGL tripeptide in vivo, three modified forms of IGL (acetyl-IGL (Ac-IGL), IGL-$NH_2$ and acetyl-IGL-$NH_2$ (Ac-IGL-$NH_2$)) were synthesized, and tested for in vitro effects on Aβ-induced inhibition of recombinant human PI4KII activity and enhancement of glutamate excitotoxicity in primary cultured hippocampal neurons. Since Ac-IGL retained its anti-Aβ activity in vitro, its effects in vivo were further investigated by intrahippocampal injection of Aβ with or without Ac-IGL in transgenic mice expressing V337M human tau protein, followed by analyses of behavior performance in the Morris water maze and histological observation of the brain.

## Materials and methods

### *Animals*

Transgenic mice expressing V337M human tau protein were kindly donated by Dr. Akihiko Takashima (Tanemura et al., 2002), Laboratory for Alzheimer's disease, the Institute of Physical and Chemical Research (RIKEN), Saitama, Japan. The mice were housed in standard cages at 3–5 animals per cage and supplied with food and water ad libitum under a 12 h/12 h light/dark cycle. The animal treatment and the experimental procedures were all based on the Guidelines for Animal Care and Use Committee at Kansai Medical University.

### *Reagents*

$A\beta_{1-42}$, $A\beta_{32-34}$ (Ile-Gly-Leu, IGL) and modified $A\beta_{32-34}$ tripeptides (Ac-IGL, IGL-$NH_2$, Ac-IGL-$NH_2$) were synthesized at Peptide Institute, Inc., Osaka, Japan. The peptide stock solutions (20 μM) for in vitro experiments were prepared in 10% dimethylsulfoxide and stored at −80 °C. These stock solutions were diluted with distilled water immediately before the in vitro experiments. The stock solution of Ac-IGL-$NH_2$ was dissolved in 100% dimethylsulfoxide because of its low solubility in water. $A\beta_{1-42}$ and Ac-IGL used in experiments in vivo were dissolved in artificial cerebrospinal fluid (aCSF: 124 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 1.25 mM $NaH_2PO_4$, 10 mM glucose, 1.3 mM $MgSO_4$, and 2.0 mM $CaCl_2$, pH 7.4). Tissue-Tek O.C.T. compound was from Sakura Finetek USA, Torrance, CA, USA; methyl green solution, Mayer's hematoxylin solution and 3,3′-diaminobenzidine tetrahydrochloride (DAB) were from Wako, Osaka, Japan; pyronin Y was from Sigma-Aldrich, St. Louis, MO, USA; and anti-cleaved caspase-3 polyclonal antibody was from CHEMICON International, Inc. Other reagents were of the highest quality commercially available.

### *In vitro experiments*

#### *Assay of recombinant human PI4KII activity*

Recombinant human phosphatidylinositol 4-kinase type IIα (PI4KIIα) was prepared by transfection of the plasmid (pGEX-KG) containing the open reading frame of human PI4KIIα into competent *Escherichia coli* DH5α cells (Toyobo, Osaka, Japan) as described previously (Wu et al., 2004). PI4KIIα activity was evaluated by measuring the phosphorylation of exogenous phosphatidylinositol (PI) using [γ-$^{32}$P] ATP (10 mCi/mmol) as a phosphate donor (Barylko et al., 2001).

#### *Cell viability analysis*

Primary cultures of rat hippocampal neurons were prepared as introduced previously (Xiong et al., 2007). The application of peptide was described in Fig. 2A. Cell viability was assessed by measuring the

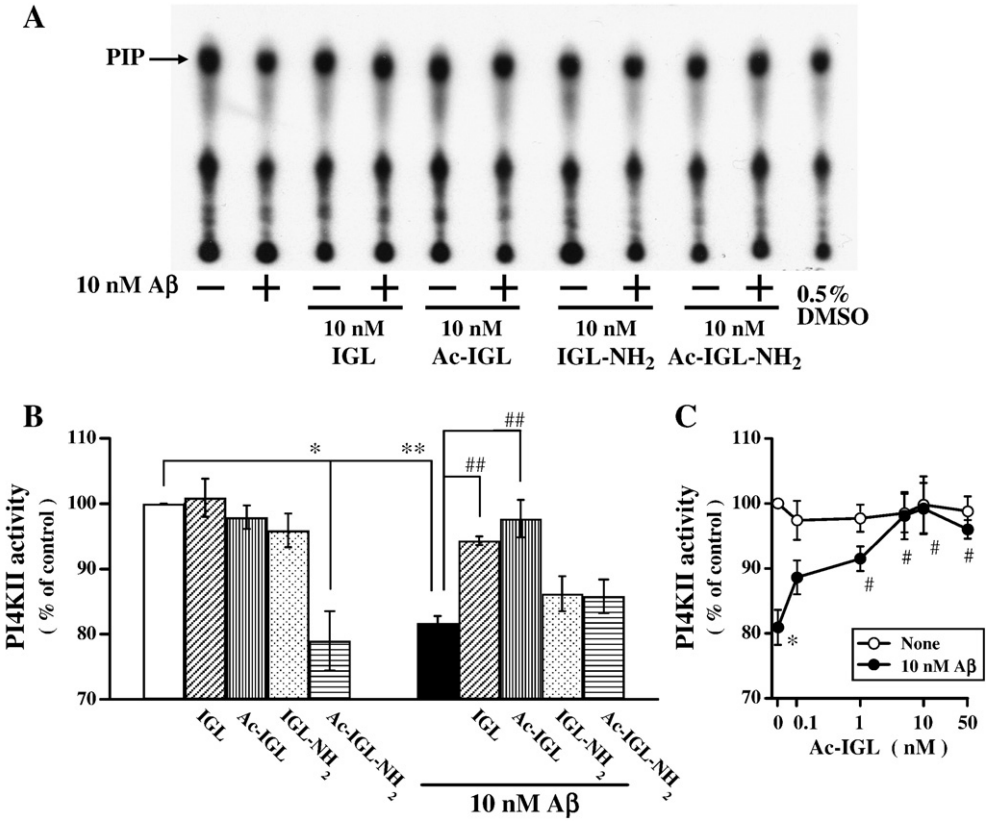


**Fig. 1.** Effects of modified IGL peptides on $A\beta_{1-42}$ induced inhibition of recombinant human PI4KII activity. PI4KII was prepared freshly and pre-incubated with or without $A\beta_{1-42}$ (final concentration, 10 nM) in the presence or absence of different modified IGL peptides for 30 min at room temperature. The kinase activity assay was initiated by the addition of [γ-$^{32}$P] ATP and $MgCl_2$ at 37 °C for 10 min, then terminated by the addition of 4 volumes of chloroform/methanol/HCl (20:40:1, v/v). (A) Typical spots of phosphatidylinositol monophosphate (PIP) as products of PI4KII activity. (B) Summary of the effects of IGL and modified IGL peptides. (C) Dose-dependent effects of 0.1–50 nM Ac-IGL on $A\beta_{1-42}$ induced inhibition of PI4KII activity. $^{*}p<0.05$, $^{**}p<0.01$ vs. control; $^{\#}p<0.05$, $^{\#\#}p<0.01$ vs. 10 nM $A\beta_{1-42}$ alone, $n=3$–4. Each bar represents the mean±S.E.M.