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Effects of peptides derived from BACE1 catalytic domain on APP processing

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ABSTRACT

One of the hallmarks of Alzheimer's disease (AD) is the deposition of β-amyloid (Aβ) peptides in neuritic plaques. Aβ peptides are derived from sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases. β-APP cleaving enzyme-1 (BACE1) has been shown to be the major β-secretase and is a primary therapeutic target for AD. We report here novel BACE1 inhibitory peptidomimetics, which are derived from catalytic domains of BACE1 themselves, instead of APP cleavage sites and are structurally modified by myristoylation in N-terminus for efficient cell permeability. The peptides not only inhibited the formation of APPβ (a soluble N-terminal fragment of APP cleaved by β-secretase), but also significantly reduced Aβ40 production. Our results suggest a new approach for identifying inhibitory agents for the treatment of AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by extracellular deposition of amyloid plaques, accumulation of intracellular neurofibrillary tangles, and neuronal cell loss in the brain [2,15]. Although the pathogenesis of AD remains unclear, it is widely accepted that extracellular accumulation of neurotoxic peptides, β-amyloids (Aβ40 and Aβ42 containing 40 and 42 amino acids, respectively) are proposed to be cause for inducing AD. Aβ peptides are derived from a sequential proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases [20].

APP is initially cleaved by β-secretase into a soluble N-terminal fragment, APPβ, and a membrane-associated C-terminal fragment, C-99. The C-99 fragment then undergoes proteolysis by γ-secretase to generate Aβ [4]. Mutations in APP or in components of the γ-secretase complex in familial forms of AD (FAD) lead to enhanced production of Aβ, particularly Aβ42 and its deposition in the neuritic plaques. Extensive efforts have been made to develop γ-secretase inhibitors to prevent the formation of Aβ. Several γ-secretase inhibitors have advanced to clinical testing; however, some concerns, have been raised about potential adverse effects of γ-secretase inhibition due to the impact on the Notch pathway [16] and the toxic effect of accumulated C-99 [9].

BACE1, a type 1 membrane-bound aspartyl protease, is the major β-secretase [7,11,18,19]. BACE1 knockout homozygote mice show a complete absence of Aβ [12] and have no reported side effects. BACE1 deficiency also rescued memory deficits and cholinergic dysfunction in transgenic mouse models of

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AD [10]. Furthermore, expression level and activity of BACE1 were found to be elevated in the brains of AD patients [10]. BACE1 is therefore considered an attractive therapeutic target for AD and may offer advantages over γ-secretase inhibition [8]. The development of β-secretase inhibitors has mainly focused on the substrate sequence of the APP β-cleavage site P4′-P4, EVKM*DAEF [1,3,5]. Despite many efforts, however, few reports of progress toward clinically useful BACE1 inhibitors have appeared.

In this study, we explore an alternative approach to inhibiting β-secretase activity. It was recently reported that protein kinase activity is selectively inhibited by short peptides derived from its catalytic domain [13]. Our work is based upon deriving short peptides from specific regions in the catalytic domain of the kinase that are implicated in kinase–substrate interactions. The substrate interacting peptides are then myristoylated in N-terminus for facilitation of membrane permeability. We tested whether this concept is also available in the design of BACE1 inhibitors based on its catalytic domain. Several peptides were designed and their effects on APP metabolism were investigated.

2. Materials and methods

2.1. Peptides

Peptides (purity > 85%) were synthesized by Fmoc solid phase peptide synthesis and purified by reverse phase-high performance liquid chromatography (Waters 2690 Separations Module, Waters, MA, USA) using Vydac C8 analytical RP column, by Peptron (Daejeon, Korea). Identity and purity were confirmed by high performance liquid chromatography mass spectroscopy (HP 1100 Series LC/MSD, Hewlett-Packard, CA, USA).

The peptides were dissolved in dimethyl sulfoxide (DMSO) at 10 mM concentration and were further diluted for the analysis.

2.2. Analyses of APPα, APPβ and full-length APP

Human embryonic kidney (HEK) 293 cells, which stably express human wild type APP695 and BACE1 (BA-3) were used for the analyses of APP metabolites, sAPPα (a soluble and extra-cellular N-terminal fragment of APP cleaved by α-secretase), sAPPβ (a soluble and extra-cellular N-terminal fragment of APP cleaved by β-secretase), intracellular APPα, intracellular APPβ, and full-length APP.

The cells were maintained in DMEM supplemented with 10% FBS. The cells were grown on 35-mm tissue culture dishes in a 5% CO_2 incubator at 37 °C, until they became 80–100% confluent. The cells then were washed once with serum-free DMEM, and serum-free DMEM with or without peptides then was added, followed by culture for 24 h. To analyze sAPPα and sAPPβ in the conditioned medium, the medium was collected, and subjected to western blot analysis. Cell extracts were prepared for intracellular APPα, APPβ, and full-length APP. The cells were harvested in cold PBS, re-suspended in a lysis buffer (50 mM Tris–HCl, pH7.6, 180 mM NaCl, 2 mM EDTA, 1% Triton X-100) and kept on ice for 30 min in the presence of protease inhibitor cocktail. After brief sonication, extracts were clarified by centrifugation at 14,000 rpm at 4 °C. Supernatant was recovered and protein concentration was determined by BCA assay. Cell extracts were subjected to western blot analysis. APPα and APPβ (intracellular and secreted form) were detected by using primary antibodies, MAB1560 (CHEMICON International Inc., CA, USA) and Rb53 (generous gift from Cephalon Inc., PA, USA) recognizing C-terminus of sAPPβ, respectively. The detection of full-length APP was performed by primary antibody MAB343 (CHEMICON International Inc., CA, USA). All blots were probed with either anti-rabbit or anti-mouse horseradish peroxidase-conjugated (Amersham Biosciences, UK) secondary antibodies and detected by chemiluminescence (ECL, Amersham Biosciences, UK).

2.3. BACE activity in vitro

For the analysis of BACE activity in vitro, the extracts of BA-3 were used as a BACE enzyme source. The cells were grown on 100-mm tissue culture dishes in a 5% CO_2 incubator at 37 °C, harvested in cold PBS, re-suspended in lysis buffer (10 mM Mes, pH6.0, 0.5% TritonX-100) and kept on ice for 15 min. After brief sonication, extracts were clarified by centrifugation at 14,000 rpm at 4 °C. Supernatant was recovered and used as BACE1 enzyme sources.

Briefly, BACE enzyme assays were carried out as follows. Peptides and the commercial BACE inhibitor, OM99-2 (H-EVN-[(2R,4S,5S)-5-amino-4-hydroxy-2,7-dimethyl-octanoyl]-A-EF-OH, Bachem, PA, USA), were pre-incubated with 20 μg cell extracts containing buffer in 20 mM sodium acetate, pH 5.0 for 10 min. β-Secretase substrate (DABCYL-VNLDAE-EDANS, Bachem, PA, USA) was then added with 20 μM and incubated for 15 min at 37 °C. At the end of the incubation, fluorescence was recorded at 360/40 and 485/20 nm as excitation and emission wavelengths, respectively (Synergy HT, BioTek, VT, USA).

2.4. Measurement of Aβ40 in conditioned medium

For the determination of Aβ40 in a conditioned medium, HEK 293 cells over-expressing human Swedish mutant APP695 and BACE1 (HBA-7 cells, generous gift from Dr. Mook-Jung Inhee) were used. Culture conditions of HBA-7 were the same as those of BA-3 except that peptides were treated for 48 h.

The amount of Aβ40 in the conditioned medium was determined by sandwich ELISA using β-amyloid [1–40] (Aβ40) Immunoassay kit (Biosource International, CA, USA).

3. Results

3.1. Design of peptides derived from BACE1 catalytic domain

The structure of the catalytic domain of human BACE1 bound to transition state inhibitors, OM99-2 (EVNLAAEF) and OM00-3 (ELDLAVEF) has been determined by X-ray crystallography [6]. The active site cleft in the catalytic domain accommodates eight subsite pockets interacting with inhibitors (Fig. 1). The regions participating in each subsite pocket are BACE1