

Selective neuronal degeneration induced by soluble oligomeric amyloid beta protein¹

HYEON-JIN KIM, SOO-CHEON CHAE,* DAE-KWON LEE,[†] BRETT CHROMY,[‡] SAM CHEOL LEE,[§] YEONG-CHUL PARK,^{||} WILLIAM L. KLEIN,[‡] GRANT A. KRAFFT,⁺⁺ AND SEONG-TSHOOL HONG^{†,2}

Research Division, Jinis Biopharmaceuticals Co., Chonju, Chonbuk, South Korea; *Genomic Research Center for Immune Disorder, School of Medicine, Wonkwang University, Iksan, Chonbuk, South Korea; [†]Institute of Cardiovascular Research and Department of Microbiology, Chonbuk National University Medical School, Chonju, Chonbuk, South Korea; [‡]Department of Neurobiology and Physiology, Northwestern University, Evanston, Illinois, USA; [§]Department of Advanced Materials Engineering, Hanlyo University, Kwangyang, Chonnam, South Korea; ^{||}Research Center of Biotechnology for Human, HumanBio Co., Iksan, Chonbuk, South Korea; and ⁺⁺Evanston Northwestern Healthcare Research Institute, Evanston, Illinois, USA

SPECIFIC AIM

Alzheimer's disease (AD) is characterized by loss of intellectual ability due to selective damage to the specific neuronal circuits in the neocortex, hippocampus, and basal forebrain cholinergic system, which play major roles in memory and cognition. The present study was undertaken to test the hypothesis that soluble amyloid beta protein (A β) has selective cytotoxicity relevant to the selective neurodegeneration observed in AD brain.

PRINCIPAL FINDINGS

1. Soluble oligomeric A β and fibrillar A β are distinguished by their structural difference

The structural differences between soluble oligomeric A β and fibrillar A β are clearly evident in images obtained by atomic force microscope (AFM). In AFM, soluble oligomers appear as globular structures <10 nm in diameter, suggesting they comprise oligomeric A β species up to hexamer as verified by SDS-PAGE. Fibrillar A β , however, shows protofibrils and fibrils as well as some aggregates that can be as large as 20 nm in diameter and several microns in length.

2. Soluble oligomeric A β is selectively toxic to CNS neuronal cells

To demonstrate the biological effects of two distinct forms of A β (1–42), we studied the cytotoxicity of soluble oligomeric A β and fibrillar A β in five different cell lines using an MTT reduction assay with A β concentrations ranging from 0.6 to 5 μ M. In NIH-3T3, SH-SY5Y, HTB186, and M059K cells, soluble oligomeric A β did not show any strong cytotoxicity in contrast to fibrillar A β , which induced significant cytotoxicity in a dose-responsive manner.

However, soluble oligomer and fibrillar A β responses were reversed in NT2. Soluble oligomeric A β toxicity in NT2 cells was much more potent than fibrillar A β at concentrations as low as 0.6 μ M, suggesting selective vulnerability of NT2 cells to soluble oligomeric A β .

3. Hippocampal formation, particularly CA1, is selectively vulnerable to soluble oligomeric A β

Organotypic brain slice assay was used to compare the neurotoxicity of soluble oligomeric A β and fibrillar A β in semi-in vivo environment (Fig. 1). Using the Live/DeadTM assay, mouse cerebral slices exposed to vehicle control exhibited background levels of dead cells and metabolically healthy neurons in subiculum (SB), cornu Ammonis (CA) fields, dentate gyrus (DG), and surrounding entorhinal cortex and temporal gyrus area (Fig. 1*a, e*). Freshly prepared monomeric A β resulted in no significant cell death as shown in the live and dead images (Fig. 1*b, f*). Exposure to soluble oligomers, however, induced cytotoxicity in the hippocampal neurons as seen in the dead (Fig. 1*c*) and live images (Fig. 1*g*). Moreover, the CA1 subfield showed a substantially higher proportion of live cells relative to the CA3 subfield. However, slices treated with fibrillar A β showed extensive neurotoxicity spread throughout the cortical explants with few regions of live neurons (Fig. 1*d, h*). Quantitative image analysis confirmed the selective vulnerability of the CA1 subfield to soluble oligomeric A β .

In full-dose response experiments ranging from 0.01 to 5 μ M, statistical analysis of slices treated with soluble

¹ To read the full text of this article, go to <http://www.fasebj.org/cgi/doi/10.1096/fj.01-0987fje>; to cite this article, use *FASEB J.* (November 1, 2002) 10.1096/fj.01-0987fje

² Correspondence: Institute of Cardiovascular Research and Department of Microbiology, Chonbuk National University Medical School, San 2–20, Kumam-Dong, Chonju, Chonbuk 561–712, South Korea. E-mail: seonghong@hotmail.com

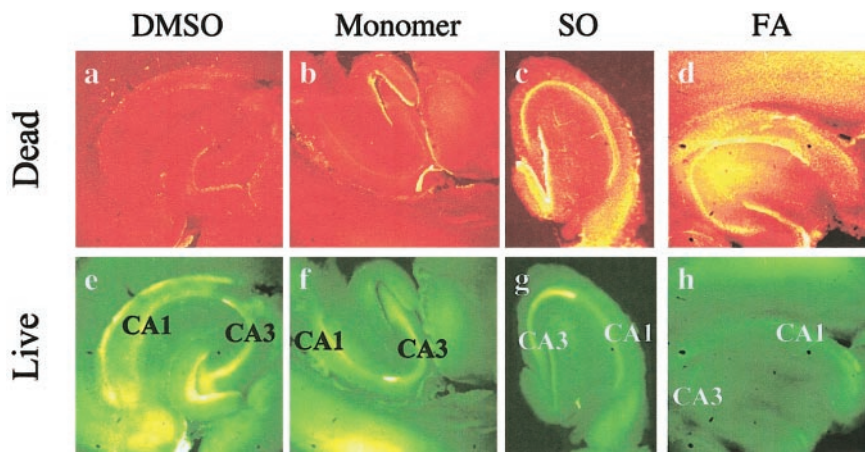


Figure 1. Selective vulnerability of the CA1 field to soluble oligomeric A β in semi-in vivo. False color-coded images of the Live/Dead (calcein/ethidium homodimer)-labeled cerebral cultures containing hippocampal formation are shown. Images were obtained after 24 h exposure to 5 μ M of A β or equivalent amount of DMSO. Representative images are shown from slices treated with DMSO control (a, e), fresh monomer (b, f), soluble oligomers (labeled as SO; c, g), or fibrillar A β (labeled as FA; d, h). The red stain (a–d) indicates cell death: membrane damage allows the entry of ethidium homodimer. Green color (e–h) is from staining with calcein, a fluorescent dye activated by intracellular esterase, indicating the presence of live, healthy neurons. CA1, regiosuperior zone of pyramidal cell field; CA3, regioinferior zone.

ase, indicating the presence of live, healthy neurons. CA1, regiosuperior zone of pyramidal cell field; CA3, regioinferior zone.

oligomers confirmed that CA1 had significantly more dead neurons than did CA3, implying the selective vulnerability of CA1 to soluble A β . In contrast, slices treated with fibrillar A β exhibited nonselective toxicity for neurons in both CA1 and CA3.

4. Resistance of cerebellum to soluble A β

We also investigated the neurotoxicity of soluble oligomeric A β and fibrillar A β in organotypic slices taken from the mouse cerebellum (Fig. 2). Consistent with all earlier toxicity results, fibrillar A β was toxic to cerebellar neurons compared with DMSO controls, which showed negligible cell death. Remarkably, soluble oligomeric A β induced very little cell death in these cerebellar slices, even at the highest A β concentrations tested.

CONCLUSIONS AND SIGNIFICANCE

A critical issue concerning the mechanism of AD is the selective regional vulnerability observed in the disease. The prevailing amyloid hypothesis of AD holds that

amyloid β protein (A β) becomes toxic when it adopts a fibrillar conformation and that fibrillar A β deposition in senile plaques (SP) causes neuronal degeneration. However, the actual neurodegeneration observed in AD brains is highly selective and regiospecific. This raises the important question of how fibrillar A β can be present throughout the brain, inducing extensive neurodegeneration in certain regions while substantially sparing neurons in other regions, such as the cerebellum. Recent studies have shown that soluble forms of A β exhibited strong neurotoxicity and that increased soluble A β can cause AD. However, the soluble A β hypothesis still does not explain the regiospecific neurodegeneration observed in AD, because soluble toxic forms of A β would be expected to be distributed throughout the brain and accessible to all types of neurons.

Here, we present evidence suggesting that the neurotoxicity induced by soluble oligomeric A β may be responsible for this selective regiospecific neurodegeneration in AD etiology. In five cell lines, fibrillar A β was toxic to all cells whereas soluble oligomeric A β induced neurotoxicity only in the CNS neuronal precursor cells. Furthermore, in organotypic cerebral slice culture,

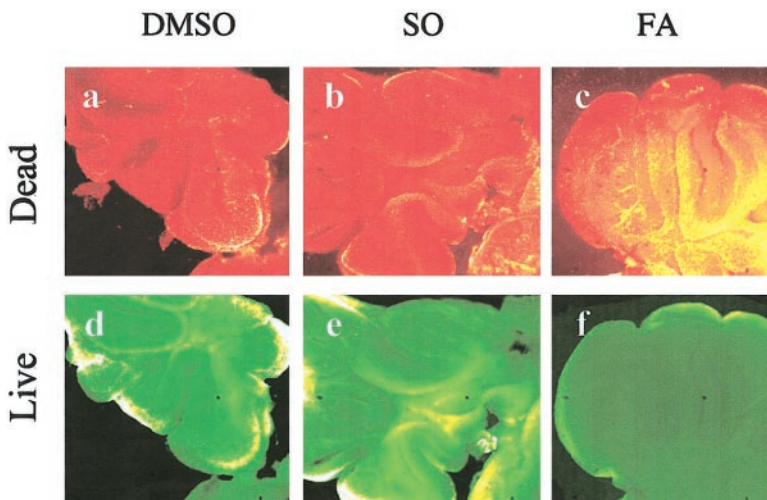


Figure 2. Selective resistances of cerebella neurons to soluble oligomeric A β in semi-in vivo. False color-coded images of the Live/Dead (calcein/ethidium homodimer)-labeled cerebellum cultures are shown. Cerebellar slices were treated with 5 μ M of A β or equivalent amount of DMSO control for 24 h and the LIVE/DEAD assay was carried out. Representative images were obtained from slices treated with DMSO control (a, d), soluble oligomeric A β (SO; b, e), or fibrillar A β (FA; c, f). A red stain (a–c) indicates cell death: membrane damage allows the entry of ethidium homodimer. Green color (d–f) is from staining with calcein, a fluorescent dye activated by intracellular esterase, indicating the presence of healthy neurons.

soluble oligomeric A β was selectively toxic to neurons in the hippocampal formation CA1, sparing the CA3 field. Several factors emphasize the selective sensitivity of hippocampal CA1 to soluble A β : CA1 is the first region to degenerate at early stages of AD and degeneration is correlated with AD severity; slice cultures has demonstrated that A β is internalized selectively and accumulated by CA1; the CA1 field exhibits an impaired LTP in response to soluble A β , thus mimicking the pathological attributes of AD. Another significant observation in this study is the selective resistance of cerebella neurons to soluble oligomeric A β . We report for the first time the selective cytotoxicity of A β -derived species to cortical neurons but not to the cerebellum. In terms of a neurodegeneration profile, the resistance of the cerebellar slices to soluble oligomeric A β in mice is consistent with the sparing of cerebellum in the brain of AD patient, providing an explanation for how soluble A β can be a potent neurotoxin in certain brain regions, yet completely spare neurons in other regions, such as the cerebellum.

Although genetic data indicate a central role for A β in the etiology of AD, the active form of the peptide that produces the pattern of neurodegeneration observed in the disease has not been identified. Selective vulnerability of hippocampal CA1 neurons and selective resistance of cerebellar neurons to soluble oligomeric A β in this study are consistent with the selective regional neurodegeneration observed in AD brain, thus challenging the relevance of A β fibrils but suggesting the pathological relevance of soluble oligomeric A β in AD etiology (**Fig. 3**). This hypothesis is supported by the following: 1) soluble A β concentrations in brain are highly correlated with severity of disease; 2) A β oligomerization is enhanced by expression of AD-linked mutations in vitro, thus connecting soluble, oligomeric A β with AD genetics; 3) soluble oligomeric A β is neurotoxic in vivo and in vitro; 4) selective increase of

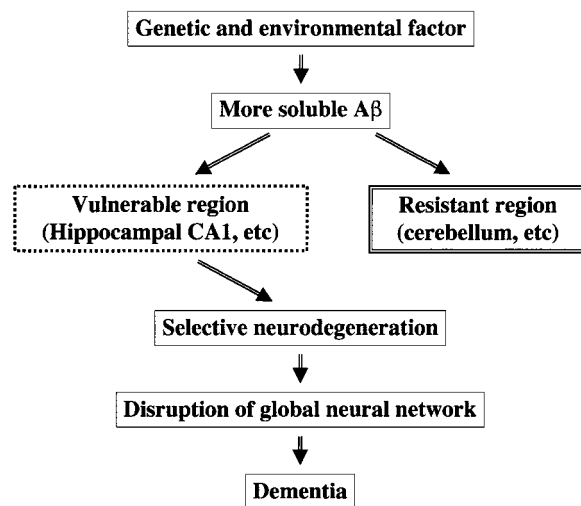


Figure 3. Hypothetical steps of the pathogenesis of Alzheimer's disease

soluble A β causes AD; 5) soluble peptide species are drawing attention in other neurodegenerative diseases that are characterized by selective vulnerability. The fibrillar SP could simply be a consequence of the local accumulation of A β fibril as surrogate markers for the disease process.

In conclusion, soluble oligomeric A β may be a missing link that can resolve an apparent contradiction in the amyloid cascade hypothesis, providing a possible explanation for the selective regional neurodegeneration that characterizes AD. Understanding the cellular mechanisms underlying a specific pathogenic process usually identifies viable molecular targets. Therefore, our observation of the selective neurotoxicity of soluble oligomeric A β to neurons involved in cognitive function may provide a new opportunity for the development of an effective AD therapy as well as elucidating the pathological mechanism of AD. FJ