

Short Communication

Possible Involvement of Small Oligomers of Amyloid- β Peptides in 15-Deoxy- $\Delta^{12,14}$ Prostaglandin J₂-Sensitive Microglial ActivationKazuyuki Takata¹, Yoshihisa Kitamura^{1,*}, Masaaki Umeki¹, Daiju Tsuchiya¹, Jun-ichi Kakimura¹, Takashi Taniguchi¹, Peter J. Gebicke-Haerter² and Shun Shimohama³¹Department of Neurobiology, Kyoto Pharmaceutical University, Kyoto 607-8412, Japan²Department of Psychopharmacology, Central Institute of Mental Health, Mannheim 68159, Germany³Department of Neurology, Graduate School of Medicine, Kyoto University, Kyoto 606-8507, Japan

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Abstract. In Alzheimer's disease, fibrillar amyloid- β (A β) peptides form senile plaques associated with microglia. However, the relationship between A β peptides and microglia is not fully understood. In this study, the incubation of A β 1-40 (A β 40) produced small oligomers, while incubation with A β 1-42 (A β 42) caused large molecular aggregates. Microglial production of nitrite, interleukin-6 and tumor necrosis factor- α was induced by A β 40, but not A β 42. This production was significantly reduced by 15-deoxy- $\Delta^{12,14}$ prostaglandin J₂, and it was completely suppressed by β -sheet breaker peptide, Leu-Pro-Phe-Phe-Asp. These results suggest that small oligomers, rather than large molecular aggregates, mediate microglial activation induced by A β peptides.

Keywords: amyloid- β peptide, small oligomer, microglial activation

One of the pathological characteristics of Alzheimer's disease (AD) is extracellular plaque formation by amyloid- β (A β) peptides associated with activated microglia (1, 2). Recent studies have shown that small oligomers or protofibrils of A β peptides, such as A β 1-40 (A β 40) and A β 1-42 (A β 42), are more neurotoxic than their corresponding monomers and mature fibrils (3, 4). Although it is known that A β peptides induce the production of nitric oxide (NO) and cytokines in microglia (5), the potency of cytokine production was not correlated with the formation of a secondary β -sheet structure (thioflavin-T fluorescence), an index of fibrillization (6). Thus, the relationship between microglia and A β forms, such as monomer, oligomers (or protofibrils) and aggregates (or fibrils), is still unclear.

It has been reported that a β -sheet breaker peptide, Leu-Pro-Phe-Phe-Asp (iA β 5), inhibits the fibrillogenesis of A β peptides both in vitro and in vivo (7). We recently found that A β 40, but not A β 42, induced the microglial production of cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), while extracellular A β 42, rather than A β 40, markedly induced

microglial phagocytosis (8). Thus, microglia responded differently to A β 40 and A β 42 under our experimental conditions, but the detailed mechanism of this difference is unclear. On the other hand, protein levels of cyclooxygenases (COX-1 and -2) and peroxisome proliferator-activated receptor- γ (PPAR- γ) have been shown to be increased in AD brains (9). Recent studies have suggested that 15-deoxy- $\Delta^{12,14}$ prostaglandin J₂ (15d-PGJ₂) and epoxyeicosatrienoic acids (EETs), which are an endogenous ligand for PPAR γ and potential candidates for endothelium-derived hyperpolarizing factor (EDHF), respectively, may act as anti-inflammatory mediators metabolized from arachidonic acid (9–12). In the present study, we examined the effects of iA β 5, 15d-PGJ₂, and EETs on microglial activation induced by A β peptides.

Mixed glial cells (mixture of astrocytes and microglia) were prepared from cerebral hemispheres of newborn Wistar rats. Rat microglial culture (over 97% pure) was then prepared as previously described (8). In our experiments, the hydrochloride (HCl) forms of A β peptides, which aggregate more readily than the trifluoroacetic acid (TFA) forms (13), were used. Since HCl forms of A β peptides form the β -sheet structure

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in phosphate-buffered saline (PBS) or cultured medium (13), we dissolved the lyophilized A β into the distilled and sterilized water as a 1 mM solution, and then 20 μ l aliquots were kept -80°C until used. Rat microglia were incubated with 10 μM A β 40 or A β 42 (AnaSpec, San Jose, CA, USA) in the presence or absence of 1–3 μM 15d-PGJ₂, 5,6-EET, 8,9-EET, 11,12-EET, or 14,15-EET (Cayman Chemical, Ann Arbor, MI, USA). In the case of incubation with β -sheet breaker, A β 40 was pre-incubated with iA β 5 (Peptide Institute, Osaka) for 1 h and then applied to the microglial culture. After treatment for 24 h, the accumulation of nitrite was measured spectrometrically (DU-640 Spectrophotometer; Beckman, Fullerton, CA, USA) using Griess reagent. Amounts of rat IL-6 and TNF- α in culture medium were determined by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's protocol (BioSource, Camarillo, CA, USA). The results are given as the mean \pm standard error of mean (S.E.M.). The statistical significance of differences was determined by an analysis of variance (ANOVA). Further statistical analysis for *post hoc* comparisons was carried out by the Bonferroni/Dunn test. In the case of *in vitro* aggregation, 3 μg of A β 40 (34.7 μM , HCl form) or A β 42 (33 μM), in the presence or absence of 4.4 μg of iA β 5 (347 μM), was incubated in PBS at 37°C for 24 h in a final volume of 20 μ l. Samples were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (20% gel using Tris-tricine buffer), and then immunoblotted with anti-A β antibody (Chemicon, Temecula, CA, USA).

Rat microglia treated with these reagents, at the

concentrations used in this study, did not show any changes in cell viability. Treatment with the HCl form of A β 40 (at 10 μM) induced nitrite accumulation and the production of IL-6 and TNF- α (Fig. 1: A–C), while the HCl form of A β 42 did not induce such microglial activation (Fig. 1). On the other hand, the TFA forms of A β 40 and A β 42 (10 μM ; Bachem, Bubendorf, Switzerland) did not markedly induce the production of these compounds under our experimental conditions (data not shown). In the *in vitro* aggregation study, although A β 42 oligomers of a moderate molecular mass were detected even before incubation, incubation of the peptides for 24 h formed a protein band with a high molecular mass (Fig. 1D) that was the aggregated form of A β 42 peptides. In contrast, the incubation of A β 40 formed small oligomers from monomers, while large molecular aggregates were not detected. Thus, small oligomers of A β peptides, but not monomer or aggregates, may induce the microglial production of NO and cytokines. In addition, the β -sheet breaker peptide iA β 5 inhibited the formation of small A β 40 oligomers (Fig. 1D).

Based on these observations, we further examined the effects of iA β 5 or the anti-inflammatory substances 15d-PGJ₂ and EETs. In rat microglial culture, nitrite accumulation and cytokine production did not occur upon treatment with either 10 μM 15d-PGJ₂ alone (data not shown) or 100 μM iA β 5 alone. The nitrite accumulation and production of IL-6 and TNF- α induced by the HCl form of A β 40 were almost completely inhibited by treatment with iA β 5 (Figs. 2 and 3). This A β 40-

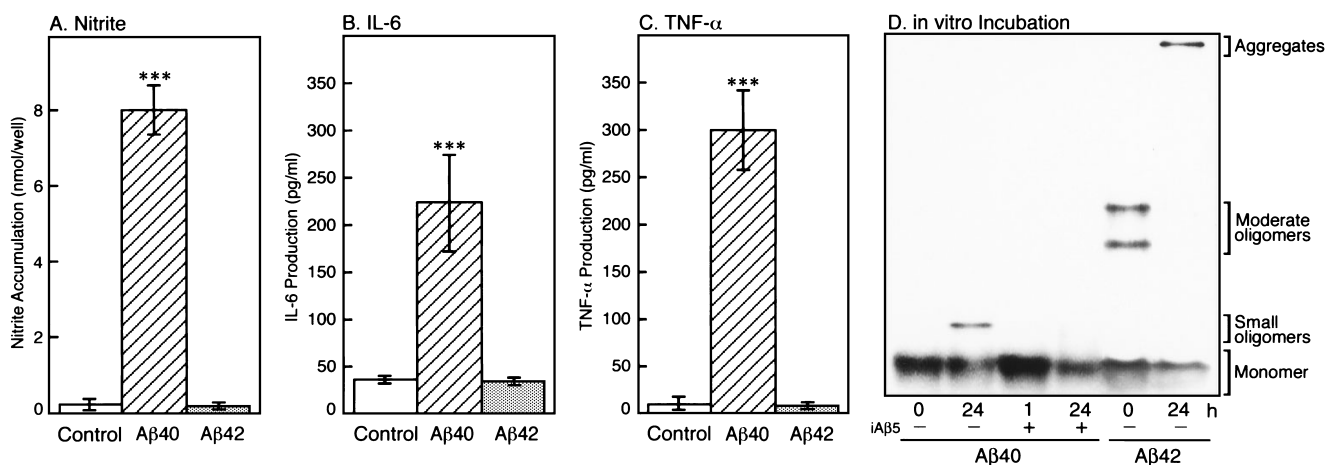


Fig. 1. Effects of A β 40 and A β 42 on nitrite accumulation and cytokine production. After treatment for 24 h with the HCl forms of A β 40 or A β 42 (at 10 μM), amounts of nitrite (A), IL-6 (B), and TNF- α (C) in rat microglial culture media were assessed. *** $P < 0.001$ vs control. D: After *in vitro* incubation with the HCl forms of A β 40 and A β 42 in the presence (+) or absence (-) of iA β 5 at 37°C for 0, 1, and 24 h, samples were subjected to immunoblot assay using anti-A β antibody. After 24 h of incubation, large molecular aggregates of A β 42 were observed, while small oligomers of A β 40 were detected. The formation of small A β 40 oligomers was inhibited by iA β 5.

induced microglial production was significantly suppressed by 15d-PGJ₂, but was not markedly inhibited by EETs, such as 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (Figs. 2 and 3).

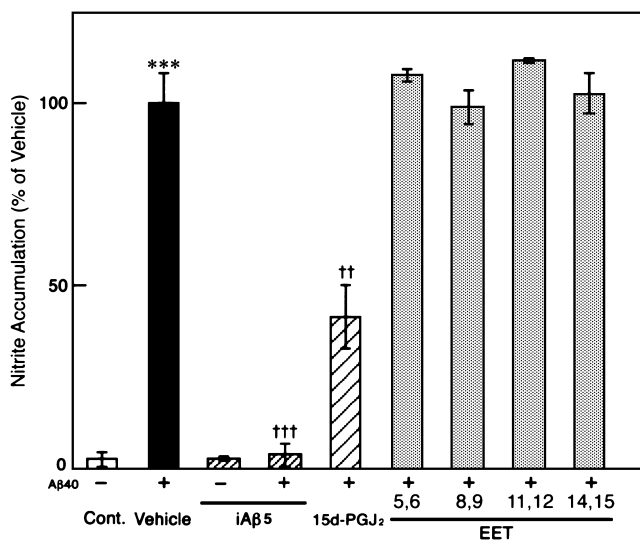


Fig. 2. Inhibition of A β 40-induced nitrite accumulation by iA β 5 or 15d-PGJ₂, but not by EETs. Rat microglia were treated with iA β 5 (at 100 μ M), 15d-PGJ₂ (at 3 μ M), or EETs (at 1 μ M) in the presence (+) or absence (-) of 10 μ M A β 40. After 24 h, the supernatant from each well was recovered and nitrite accumulation was measured. Each value is the mean \pm S.E.M. of three determinations. *** P <0.001 vs control. †† P <0.01, ††† P <0.001 vs vehicle.

EETs, which are products of cytochrome P450 epoxygenases, have EDHF-like function and anti-inflammatory properties (12). Among them, 11,12-EET has been shown to have the strongest inhibitory effect, which was apparent even at 0.1 μ M in endothelial cells (12). It has been thought that EET-induced anti-inflammation is mediated through the inhibition of transcription factor NF- κ B and I κ B kinase (12), similar to 15d-PGJ₂ (11). In this study, 15d-PGJ₂ inhibited A β 40-induced microglial activation, like the inhibition of lipopolysaccharide-induced inflammation in peripheral monocytes and macrophages (10, 11). On the other hand, EETs did not influence nitrite accumulation or cytokine production induced by A β 40. These results suggest that A β 40-induced microglial activation is sensitive to 15d-PGJ₂, but not EETs.

Previous in vitro studies showed that when TFA forms of A β peptides were incubated for a long time, they (also known as 'aged' A β) exhibited greater neurotoxic effects than freshly prepared 'new' peptides (14). Therefore, it is thought that mature fibrils of A β peptides may cause neurotoxicity. However, there are several discrepancies; e.g., in the brain of double-transgenic mouse carrying both mutant presenilin-1 and A β precursor protein transgenes (PS/APP mouse), although the marked production of A β 40 and A β 42 and the accelerated formation of A β deposits occur, global neuronal loss is not detected (15). In contrast, recent studies have suggested that small oligomers or

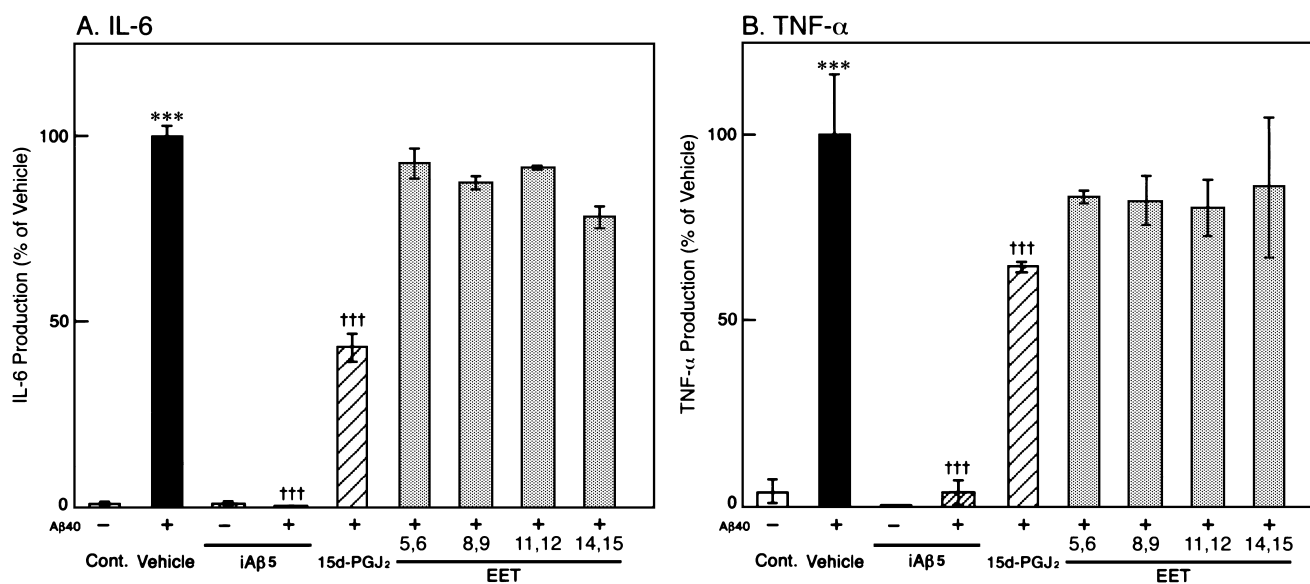


Fig. 3. Inhibition of A β 40-induced cytokine production by iA β 5 or 15d-PGJ₂, but not by EETs. Rat microglia were treated with iA β 5 (at 100 μ M), 15d-PGJ₂ (at 3 μ M), or EETs (at 1 μ M) in the presence (+) or absence (-) of 10 μ M A β 40. After 24 h, amounts of IL-6 (A) and TNF- α (B) were determined using a rat ELISA kit. Each value is the mean \pm S.E.M. of three determinations. *** P <0.001 vs control. ††† P <0.001 vs vehicle.

protofibrils of A β peptides were more neurotoxic than monomers and fibrils (3, 4). In microglial culture, although A β peptides induced cytokine production, this potency was not correlated with β -sheet formation (6). A β 42 is more readily fibrillated than A β 40, and their HCl forms facilitate such aggregation compared to their TFA forms (13). Therefore, in this study, we used the HCl forms of A β 40 and A β 42. In vitro incubation of A β 40 induced the formation of small oligomers, while the incubation of A β 42 formed large molecular aggregates. The iA β 5 inhibited the formation of small A β 40 oligomers. In addition, A β 40-induced nitrite accumulation and production of IL-6 and TNF- α were almost completely inhibited by iA β 5. Since iA β 5 may break oligomers or protofibrils of A β peptides (7), this treatment did not induce microglial production of NO, IL-6, and TNF- α . These observations suggest that 15d-PGJ₂-sensitive microglial activation induced by A β peptides may be mediated by small oligomers (or protofibrils) of A β peptides rather than monomers and aggregates (or fibrils).

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