学 位 論 文 の 要 旨

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学 位 論 文 名　Evaluation of the Inhibitory Effect of Docosahexaenoic Acid and Arachidonic Acid on the Initial Stage of Amyloid β_{1-42} Polymerization by Fluorescence Correlation Spectroscopy

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論 文 内 容 の 要 旨

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the deposition of amyloid β (Aβ) peptides in neuritic plaques and neurofibrillar tangles in the affected brain regions. $A\beta_{1-42}$ constitutes the foremost component of neuritic plaques and tangles of the affected brains and plays an important role in neurobehavioral impairments in AD. Formation of fibers is central to AD pathogenesis.

Docosahexaenoic acid (DHA) is the most abundant n-3 polyunsaturated fatty acid (PUFA) in the mammalian brain, and deficiency of this PUFA is associated with memory impairment in AD model rats and AD patients. Oral administration of DHA decreases the amyloid burden in the brains of AD model animals, with a concomitant *in vitro* inhibition of the amyloid fibril formation, by acting at various stages of polymerization. Thus, DHA is suggested to be a potent therapeutic and preventive agent against Aβ-induced AD.

In this experiment, we have used fluorescence correlation spectroscopy (FCS) to delineate the temporal resolution of DHA-induced mechanisms of inhibition of amyloid fibrillation. In addition, the effects of other PUFAs such as eicosapentaenoic acid (EPA), a precursor for DHA, and arachidonic acid (AA), the abundant n-6 PUFA in the brain,

on amyloid polymerization have been studied using this technique. Furthermore, we examined whether metabolites of DHA, including neuroprotectin D1 (NPD1) and resolvin D1 (RvD1), and didosahexaenoyl glycerol (diDHA), inhibited $A\beta_{1-42}$ polymerization.

MATERIALS AND METHODS

DHA, EPA, AA, NPD1 and RvD1 dissolved in ethanol were stored at −80°C, diDHA dissolved in chloroform was stored at −30°C. On the day of use, DHA , EPA, AA, and diDHA were mixed with assembly buffer at a final concentration of 20 μM, and NPD1 and RvD1 were mixed at a final concentration of 50 - 500 nM.

FCS is a correlation analysis of fluctuations in the fluorescence intensity of fluorescent compounds excited by a focused laser beam in a very tiny space, i.e., the so-called confocal volume. The fluorescence intensity fluctuates because of Brownian motion of the fluorescent particles. This analysis gives the average number of fluorescent particles and average diffusion time when particles are passing through the tiny confocal volume.

In this experiment, the FCS measurements were performed on a Fluoro Point Light (Olympus) at room temperature using the on-board 543-nm helium/neon laser at a laser power of 100 μW for excitation. Fluorescently labeled $A\beta_{1-42}$, 5-carboxytetramethylrhodamine (TAMRA) $A\beta_{1-42}$, dissolved in 1% NH_4OH was stored at −30°C. On the day of use, it was re-dissolved in assembly buffer at 1 nM, with or without DHA, EPA, AA, NPD1, RvD1 and diDHA, and quickly mixed with 10 μM non-labeled $A\beta_{1-42}$. The measurements were performed in a sample volume of 50 μL in a 384-well glass-bottomed microplate. The samples were sequentially and automatically loaded into the device, the optical system of which was also automatically adjusted for each measurement. Initially, the samples were subjected to FCS measurement at zero time. Afterward, the samples were incubated at 37°C for 1 h, followed by a second reading using the Fluoro Point Light device. All experiments were performed under identical conditions, with a data acquisition time of 10 s per measurement, and measurements were repeated five times per sample.

RESULTS AND DISCUSSION

In these experiments, using FCS, we examined the effect of PUFAs, such as DHA, EPA and AA, diDHA, and the DHA metabolites NPD1 and RvD1, on $A\beta_{1-42}$

polymerization within 1 h of the start of fibrillation. FCS enabled us to investigate whether the examined compounds had the inhibitory effects on the initial stage of $A\beta_{1-42}$ polymerization by measuring the diffusion time. DHA inhibited $A\beta_{1-42}$ polymerization in a concentration-dependent manner. 20 μM DHA decreased the $A\beta_{1-42}$ diffusion time by 28% within 1 h, suggesting that DHA had the inhibitory effect on the initial stage of $A\beta_{1-42}$ polymerization. AA also reduced the $A\beta_{1-42}$ diffusion time by 31% within 1 h, suggesting that AA also had the inhibitory effect on $A\beta_{1-42}$ polymerization. On the other hand, EPA did not reduce the $A\beta_{1-42}$ diffusion time within 1 h, indicating that EPA did not affect $A\beta_{1-42}$ polymerization. Previous studies reported that EPA had the beneficial effects on AD and this result suggested that these effects of EPA occurred after EPA was transformed into DHA. According to molecular dynamics simulations and computational analyses, DHA, EPA and AA form ball-shaped curves. DHA and AA have six and four double bonds, respectively, in their carbon skeleton, and these double bonds may face each other. EPA, which has five double bonds, may have double bonds that do not face each other while it interacts with amyloid peptides. These results and possibilities might relate to the differences between the inhibitory effects of DHA, AA and EPA on $A\beta_{1-42}$ polymerization.

The DHA metabolites including NPD1 and RvD1 are produced during inflammation. Inflammation also contributes to AD pathogenesis. In this experiment, NPD1 and RvD1 did not decrease the $A\beta_{1-42}$ diffusion time, indicating that NPD1 and RvD1 did not affect $A\beta_{1-42}$ polymerization. These results suggested that DHA itself inhibited $A\beta_{1-42}$ polymerization before being converted to its metabolites, including NPD1 and RvD1. High concentration of DHA is found in retinal rod outer segment, sperm, and synaptosomes, and didocosahexaenoyl phospholipid species have been isolated from several tissues. In this experiment, diDHA did not decrease the $A\beta_{1-42}$ diffusion time, indicating that diDHA did not affect $A\beta_{1-42}$ polymerization. These results suggested that free DHA, but not didocosahexaenoyl glycerol, exerted the inhibitory effect on $A\beta_{1-42}$ polymerization.

CONCLUSION

Using FCS, we showed that DHA and AA inhibit $A\beta_{1-42}$ polymerization within short period of time. This provides new insight into the function of DHA and AA at the initial stage of $A\beta_{1-42}$ polymerization. Finally, the results of the present study demonstrate that continuous intake of DHA might delay the onset and progression of AD initiated by the earlier deposition of $A\beta_{1-42}$ fibers.