

# 学位論文の要旨

氏名 三輪 耕治

- 学位論文名 Evaluation of the Inhibitory Effect of Docosahexaenoic Acid and Arachidonic Acid on the Initial Stage of Amyloid  $\beta_{1-42}$  Polymerization by Fluorescence Correlation Spectroscopy
- 発表雑誌名 Advances in Alzheimer's Disease  
(巻, 初頁~終頁, 年) (in press)
- 著者名 Koji Miwa, Michio Hashimoto, Shahdat Hossain, Masanori Katakura, Osamu Shido

## 論文内容の要旨

### INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the deposition of amyloid  $\beta$  ( $A\beta$ ) 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  $A\beta$ -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 $\beta$ <sub>1-42</sub> polymerization.

## **MATERIALS AND METHODS**

DHA, EPA, AA, NPD1 and RvD1 dissolved in ethanol were stored at  $-80^{\circ}\text{C}$ , diDHA dissolved in chloroform was stored at  $-30^{\circ}\text{C}$ . On the day of use, DHA, EPA, AA, and diDHA were mixed with assembly buffer at a final concentration of 20  $\mu\text{M}$ , 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  $\mu\text{W}$  for excitation. Fluorescently labeled A $\beta$ <sub>1-42</sub>, 5-carboxytetramethylrhodamine (TAMRA) A $\beta$ <sub>1-42</sub>, dissolved in 1%  $\text{NH}_4\text{OH}$  was stored at  $-30^{\circ}\text{C}$ . 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  $\mu\text{M}$  non-labeled A $\beta$ <sub>1-42</sub>. The measurements were performed in a sample volume of 50  $\mu\text{L}$  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  $37^{\circ}\text{C}$  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 $\beta$ <sub>1-42</sub>

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 $\beta$ <sub>1-42</sub> polymerization by measuring the diffusion time. DHA inhibited A $\beta$ <sub>1-42</sub> polymerization in a concentration-dependent manner. 20  $\mu$ M DHA decreased the A $\beta$ <sub>1-42</sub> diffusion time by 28% within 1 h, suggesting that DHA had the inhibitory effect on the initial stage of A $\beta$ <sub>1-42</sub> polymerization. AA also reduced the A $\beta$ <sub>1-42</sub> diffusion time by 31% within 1 h, suggesting that AA also had the inhibitory effect on A $\beta$ <sub>1-42</sub> polymerization. On the other hand, EPA did not reduce the A $\beta$ <sub>1-42</sub> diffusion time within 1 h, indicating that EPA did not affect A $\beta$ <sub>1-42</sub> 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 $\beta$ <sub>1-42</sub> polymerization.

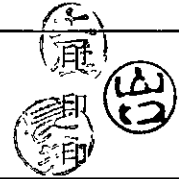
The DHA metabolites including NPD1 and RvD1 are produced during inflammation. Inflammation also contributes to AD pathogenesis. In this experiment, NPD1 and RvD1 did not decrease the A $\beta$ <sub>1-42</sub> diffusion time, indicating that NPD1 and RvD1 did not affect A $\beta$ <sub>1-42</sub> polymerization. These results suggested that DHA itself inhibited A $\beta$ <sub>1-42</sub> 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 $\beta$ <sub>1-42</sub> diffusion time, indicating that diDHA did not affect A $\beta$ <sub>1-42</sub> polymerization. These results suggested that free DHA, but not didocosahexaenoyl glycerol, exerted the inhibitory effect on A $\beta$ <sub>1-42</sub> polymerization.

## **CONCLUSION**

Using FCS, we showed that DHA and AA inhibit A $\beta$ <sub>1-42</sub> polymerization within short period of time. This provides new insight into the function of DHA and AA at the initial stage of A $\beta$ <sub>1-42</sub> 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 $\beta$ <sub>1-42</sub> fibers.

論文審査及び最終試験又は学力の確認の結果の要旨

①・乙	氏名	三輪 耕治
学位論文名	Evaluation of the Inhibitory Effect of Docosahexaenoic Acid and Arachidonic Acid on the Initial Stage of Amyloid $\beta_{1-42}$ Polymerization by Fluorescence Correlation Spectroscopy	
学位論文審査委員	主査	土屋 美加子
	副査	山口 修平
	副査	長井 篤



論文審査の結果の要旨

Alzheimer's disease (AD) 患者の脳に見られる老人斑は amyloid  $\beta$  ペプチド ( $A\beta$ ) の凝集、沈着によるものであり、このような変化が神経変性、神経細胞死の原因となる。docosahexaenoic acid (DHA) は、大脳皮質、灰白質のリン脂質に多く含まれるが、AD 患者脳での減少とともに、AD 発症抑制効果や  $A\beta$  凝集抑制作用が報告され、その AD に対する予防効果が注目されている。申請者は、DHA の  $A\beta$  凝集抑制機序解明の一環として、 $A\beta_{1-42}$  を用いてその *in vitro* における重合に及ぼす DHA の効果を、Fluorescence Correlation Spectroscopy (FCS) を用いて検討し、DHA および arachidonic acid は  $A\beta_{1-42}$  monomer の重合を抑制するが、DHA 前駆体である eicosapentaenoic acid や DHA の代謝産物、あるいは DHA の glycerol ester にはそのような効果がないことを明らかにし、遊離 DHA が重合初期段階に対しても抑制効果をもつことを初めて示した。この研究は DHA の  $A\beta$  凝集に及ぼす作用について新たな知見を加えるものであり、DHA の AD に対する予防効果の機序解明に寄与する論文であると判断した。

最終試験又は学力の確認の結果の要旨

申請者は、DHA の *in vitro* における  $A\beta_{1-42}$  重合に及ぼす影響を FCS を用いて検討し、この物質が  $A\beta_{1-42}$  重合初期にも抑制的に働くことを初めて明らかにした。関連知識ならびに質疑に対する応答も十分であり、学位授与に値すると認める。  
(主査：土屋美加子)

申請者は AD の原因と考えられている  $A\beta_{1-42}$  の重合の初期段階において、DHA が抑制作用を及ぼすことを *in vitro* で明らかにした。今後の AD の予防に繋がる研究成果で、豊富な関連知識も有しており学位授与に値すると判断する。  
(副査：山口修平)

申請者は、AD において神経細胞死の主な原因とされる  $A\beta_{1-42}$  の重合化過程について FCS を用いて分析した。その結果、DHA および arachidonic acid が選択的に  $A\beta_{1-42}$  重合化を抑制することを証明し、機序について考察した。結果の解釈は的確で、関連知識も豊富であり、学位授与に値すると認める。  
(副査：長井篤)

(備考) 要旨は、それぞれ400字程度とする。