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Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid β-infused rats relates to the decreases of amyloid β and cholesterol levels in detergent-insoluble membrane fractions

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Abstract

We investigated the effects of dietary administration of docosahexaenoic acid (DHA; C22:6n-3) on the levels of amyloid β (Aβ) peptide (1–40) and cholesterol in the nonionic detergent Triton 100×-insoluble membrane fractions (DIFs) of the cerebral cortex and, also, on learning-related memory in an animal model of Alzheimer's disease (AD) rats infused with Aβ peptide (1–40) into the cerebral ventricle. The infusion increased the levels of Aβ peptide and cholesterol in the DIFs concurrently with a significant increase in reference memory errors (measured by eight-arm radial-maze tasks) compared with those of vehicle rats. Conversely, the dietary administration of DHA to AD-model rats decreased the levels of Aβ peptide and cholesterol in the DIFs, with the decrease being more prominent in the DHA-administered rats. Regression analysis revealed a significant positive correlation between Aβ peptide and each of cholesterol, palmitic acid and stearic acid, and between the number of reference memory errors and each of cholesterol, palmitic, stearic and oleic acid; moreover, a significant negative correlation was observed between the number of reference memory errors and the molar ratio of DHA to palmitic plus stearic acid. These results suggest that DHA-induced protection of memory deficits in AD-model rats is related to the interactions of cholesterol, palmitic acid or stearic acid with Aβ peptides in DIFs where DHA ameliorates these interactions.

Keywords: Amyloid β peptide; Detergent-insoluble membrane fraction; Docosahexaenoic acid; Learning ability; Alzheimer's disease model rats

1. Introduction

The molecular event of the deposition of amyloid beta (Aβ) peptide (1–40) or Aβ peptide (1–42) in the brains of Alzheimer's disease (AD) subjects and the consequent loss of cognition is not clearly understood. The process is believed to involve complicated actions of β- and γ-secretase on their substrate amyloid precursor protein (APP) in membrane lipid environments; for example, the APP is subjected to sequential cleavage of β- and γ-secretase, a prerequisite to intramembrane events that result in the production of Aβ peptides (1–40)/(1–42) that act as seeds of amyloidogenesis [1]. Recent evidence suggests that the seeding process correlates the amyloid peptides with the cholesterol content of the so-called lipid rafts or detergent-resistant specialized membrane domains [2]. In vitro studies with human neuroblastoma cells have also shown that these specialized membrane domains are highly rich in cholesterol and glycosphingolipids and play an important role in cellular trafficking and signal transduction cascades [3]. Moreover, the decreased prevalence of AD is associated with treatment by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors [4]. Thus, interest has focused on the initial localization of amyloid peptides in cholesterol-rich membrane domains.

The search for agents capable of protecting against amyloidogenesis and associated cognitive loss by dietary intervention has been one of the most interesting aspects of AD research. Therefore, the effects of dietary docosahexaenoic acid (DHA; C22:6n-3) on Aβ peptides and cholesterol in

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detergent insoluble membrane fractions (DIFs) could be of special significance, especially since DHA reduces cholesterol in both neuronal [5] and non-neuronal cells [6]. On the other hand, dietary administration of DHA protects against [7] and ameliorates [8] the impairment of memory learning induced by the infusion of Aβ peptide (1–40) into the rat cerebral ventricle, suggesting that DHA plays an important role in the neurochemical basis of memory. DHA exerts antioxidative effects on brain neurons [7–11], and a variety of antioxidants exhibits anti-AD pathology [12]. Epidemiological studies also suggest that AD is less frequent in populations that consume DHA [13]; however, these mechanisms have remained largely unknown. The aim of this preliminary investigation was, therefore, to investigate whether Aβ peptide (1–40) infusion into the rat cerebral ventricle affects the levels of cholesterol and Aβ peptide (1–40) in DIFs and, also, whether the effects of dietary DHA on these levels are associated with memory-related learning ability in AD model rats.

2. Materials and methods

2.1. Animals and diet

Rats were provided for and killed in accordance with the procedures outlined in the *Guidelines for Animal Experimentation of Shimane Medical University* (Shimane, Japan), compiled from the *Guidelines for Animal Experimentation of the Japanese Association for Laboratory of Animal Science.* Wistar rats (generation 1, G1) (*Jcl*: Wistar; Clea Japan Co., Osaka, Japan) were housed in a room under controlled temperature (23±2 °C), relative humidity (50±10%) and light–dark cycles (light: 0800 to 2000 h: dark: 2000 to 0800 h), and provided with a fish-oil-deficient pellet diet (F-1®; Funabashi Farm, Funabashi, Japan) and water ad libitum. The fatty acid composition of the F-1® is shown in Table 1. The inbred third generation (G3) male rats [n=31; 20 weeks old; 384±5.3 g body weight (BW)], fed the same F1 diet, were randomly divided into 4 groups: a vehicle group (n=7), an Aβ peptide (1–40)-infused group (Aβ group) (n=7), an Aβ+DHA group (n=8) and a DHA group (n=9).

2.2. Surgery for the preparation of Aβ-infused AD model rats

The formation of neurofibrillary tangles and neuritic plaques of amyloid peptides such as Aβ peptide$_{(1–40)}$ and neuronal loss are hallmarks of AD [14], and we [7,8] and others [14–16] have previously reported that the infusion of Aβ peptide (1–40) into the rat cerebral ventricle induces learning impairment, and neuronal and morphological degeneration. The surgical techniques for preparing Aβ-infused rats were, therefore, essentially the same as those described [7,8]. Briefly, each rat was anesthetized with sodium pentobarbital (50 mg/kg BW i.p.). The skull was exposed and two holes (right and left, relative to the bregma; 0.8 mm posterior, 1.4 mm lateral) were drilled according to the atlas of Paxinos and Watson [17] using a stereotaxic frame (Narishige, Tokyo, Japan). A solvent of 35% (v/v) acetonitrile plus 0.1% (v/v) trifluoroacetic acid (pH 2.0) was used as the vehicle for Aβ peptide (1–40) (Peptide Inst., Osaka, Japan). Since a small amount of $AlCl_3$ facilitates the aggregation of Aβ peptide in vitro, and since the method has limited reproducibility without $AlCl_3$, we used 0.5 μg $AlCl_3$ (in 5 μL, intracerebroventricularly, 1 μL/min) before implanting the osmotic pump for continuous infusion of Aβ. This procedure greatly improved reproducibility and reliability in producing an animal model of AD with impaired memory. A mini-osmotic pump (alzet 2002; Durect Co., Cupertino, CA, USA), containing either Aβ peptide (1–40) solution or the vehicle alone was quickly implanted into the backs of the rats. The outlet of the pump was inserted 3.5 mm into the left ventricle and attached to the skull with screws and dental cement. The osmotic pump contained 234±13.9 μL Aβ solution. The infusion rate was 0.56 μL/h, and the total amount infused was approximately 4.9–5.5 nmol/L Aβ.

Table 1
Fatty acid composition (mol%) of a fish-oil-deficient pellet diet

Myristic acid $_{C14:0}$	0.09±0.09
Palmitic acid $_{C16:0}$	15.6±0.53
Palmitoleic acid $_{C16:1,\ n-7}$	ND
Stearic acid $_{C18:0}$	5.41±0.09
Oleic acid $_{C18:1,\ n-9}$	21.1±0.17
Linoleic acid $_{C18:2,\ n-6}$	52.4±0.80
Linolenic acid $_{C18:3,\ n-3}$	4.49±0.13
Arachidic acid $_{C20:0}$	0.14±0.13
Eicosenoic acid $_{C20:1,\ n-9}$	0.33±0.15
Arachidonic acid $_{C20:4,\ n-6}$	ND
Eicosapentaenoic acid $_{C20:5,\ n-3}$	0.06±0.06
Docosapentaenoic acid $_{C22:5,\ n-3}$	ND
Docosahexaenoic acid $_{C22:6,\ n-3}$	ND
Tetracosanoic acid $_{C24:0}$	0.11±0.07

Values are means±S.E.M. of quadruplicate determinations; ND: not detected.

2.3. Radial maze-related memory test

Learning-related behavior was assessed using an eight-arm radial maze (Toyo Sangyo, Toyama, Japan) to test whether memory was impaired as described previously [8,18–20]. Briefly, 4 weeks after the implantation of the mini-osmotic pump, the rats, maintained under a food-deprivation schedule, were trained to acquire a reward (food pellet) at the end of each of four arms of an eight-arm radial maze. The performance involved two parameters of memory-related function, namely, reference memory error (RME), entry into unbaited arms; and working memory error (WME), repeated entry into arms that had already been visited and obtaining the rewards within a trial. Thus, the higher the number of RME, the worse the learning ability, and vice versa. Each rat was given two daily trials, 6 days/week for a total of 2.5 weeks. The DHA and Aβ+DHA groups were then orally fed DHA-95E (300 mg/kg BW/day, an ethyl ester all-*cis*-4,7,10,13,16,19-docosahexaenoate with purity greater than 95%; Harima Chemicals, Tokyo, Japan) gently emulsified in a 5% (w/v) gum Arabic solution in ice-cold water before administration; the vehicle and Aβ groups were fed an equal volume of gum Arabic solution only. The total volume of gum Arabic solution administered with DHA was 0.5–0.8 mL. Seven weeks after starting the administration of DHA, the rats were tested for learning ability using the 8-arm radial maze for a total of 5 weeks, to assess the effect of dietary DHA on the impairment of learning ability.

2.4. Preparation of brains

After completing the behavioral studies, the rats were anesthetized with sodium pentobarbital (65 mg/kg BW, i.p.), and the blood was collected; the cerebral cortex was separated from the whole brain on ice, blotted gently with filter paper to remove blood and extraneous tissue fragments, then flash-frozen with liquid N_2 and stored at −80 °C until use.

2.5. Preparation of detergent insoluble membrane fractions (DIFs)

DIFs were prepared as previously described [21] with minor modifications. Cortical tissues (80–120 mg) were transferred to a capsule precooled in liquid N_2, crushed with an amalgam mixer (UT-1600, Sharp, Osaka, Japan) and suspended in 1.0 mL of ice-cold Tris–saline (50 mM Tris–HCl, pH, 7.6, 0.15 M NaCl) buffer containing 1% (v/v) Triton X-100 and the following protease inhibitors: 1.0 μM phenylmethylsulphonile fluoride, 10 μg/mL leupeptin, 1.0 μg/mL papstatin and 10 μg/mL aprotinin. The homogenate was centrifuged at 100,000×g for 30 min and the supernatant was used for measuring detergent-soluble Aβ peptide (1–40). The pellets were washed with the MES-buffered saline (25 mM MES, pH 6.5 and 0.15 M NaCl) containing 1% (v/v) Triton X-100 and various protease inhibitors (1.0 μM phenylmethylsulphonile fluoride, 10 μg/mL leupeptin, 1.0 μg/mL papstatin and 10 μg/mL aprotinin), and used as DIFs. After washing, the pelleted DIFs were initially suspended by vigorous mixing in a small volume of 6.0 M guanidine hydrochloride in 50 mM