

Neuroprotective and Ameliorative Actions of Polyunsaturated Fatty Acids Against Neuronal Diseases: Beneficial Effect of Docosahexaenoic Acid on Cognitive Decline in Alzheimer's Disease

Michio Hashimoto^{1,*} and Shahdat Hossain^{1,2}

¹Department of Environmental Physiology, Shimane University Faculty of Medicine, Izumo, Shimane 693-8501, Japan

²Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka, Bangladesh

Received November 16, 2010; Accepted March 14, 2011

Abstract. Docosahexaenoic acid (DHA, C22:6 n-3), the most abundant n-3 polyunsaturated fatty acid in the brain, is essential for brain growth and development. Recent evidence has indicated the potential health benefits of DHA for managing Alzheimer's disease (AD). For example, dietary administration of DHA considerably protects against and ameliorates the impairment of learning ability in amyloid-beta ($A\beta$)₁₋₄₀-infused AD-model rats, with concurrent increases in DHA levels and decreases in the levels of lipid peroxide and reactive oxygen species in the cortico-hippocampal tissues. In addition, dietary DHA helps in eliminating the amyloid burden from the brains of AD-model rats. In vitro studies have revealed that DHA substantially inhibits $A\beta$ fibrillation. Furthermore, DHA reduces amyloid-induced toxicity in cell culture. These in vitro data support the hypothesis that DHA can ameliorate the cognitive deficits of AD in vivo by limiting $A\beta$ polymerization in the brains. Therefore, it might be a useful therapeutic agent to prevent and/or delay cognitive impairment in mild cases of AD.

Keywords: Alzheimer's disease, amyloid fibrillation, docosahexaenoic acid, neurogenesis, neuronal toxicity, oxidative stress, polyunsaturated fatty acid

1. Introduction

In the biosphere, docosahexaenoic acid (DHA, C22:6 n-3) is primarily synthesized *de novo* by photosynthetic and heterotrophic microalgae, which then becomes highly enriched as a major polyunsaturated fatty acid (PUFA) in the marine food chain. It is also synthesized through the elongation and desaturation of eicosapentaenoic acid (EPA, C20:5 n-3) or the elongation of α -linolenic acid (ALA, C18:3 n-3), which is particularly abundant in chia and flax plants (1). DHA is also the most abundant n-3 PUFA in the brain, and about 30% – 40% of all esterified fatty acids in neural plasma membrane phospholipids consist of DHA (2). In the brain, endothelial cells lining the cerebrovasculature and astroglial support cells, but not neurons, can synthesize

DHA from ALA and other n-3 precursor fatty acids (3); however, whether this synthesis contributes substantially to the total brain DHA content is not clear.

During the growth spurt of the central nervous system (CNS) after birth, DHA rapidly accretes in the brain (4). It is an absolute prerequisite for the development of the human CNS (5, 6) and continuous maintenance of brain cell function, implying that DHA is essential for human mental health (7) and intellectual evolution (8, 9). For example, DHA deficiency produces marked deterioration such as cognitive impairment (10), retarded visual acuity (11), and various other neurological disorders (12, 13). Recent studies have indicated that this PUFA plays a role in cognitive development, learning ability, neural membrane plasticity, and synaptogenesis, all of which are involved in synaptic transmission and the well-being of normal brain functions (Table 1).

Alzheimer's disease (AD), a neurodegenerative disorder characterized by progressive cognitive and memory decline, is the most frequent form of dementia (14). Its

*Corresponding author. michio1@med.shimane-u.ac.jp
Published online in J-STAGE on May 21, 2011 (in advance)
doi: 10.1254/jphs.10R33FM

Table 1. Roles of docosahexaenoic acid in the brain

1	Learning and memory
2	Gene expression
3	Neurotransmitter release
4	Blood brain barrier
5	Immunity and inflammation
6	Enzymes, ion channels, and receptors
7	Adhesion molecule
8	Apoptosis
9	Neurogenesis
10	Antioxidative potential

This table was arranged from Ref. 122.

cause is still unknown and it has no cure; therefore, efforts directed at its prevention are needed. The prospect of reducing the risk of AD by preventative strategies such as diet or lifestyle modification is highly favorable. Epidemiological studies have shown that consumption of select fats and antioxidants such as vitamins E and C lowers the risk of AD (10, 15). In particular, growing evidence has shown that moderate fish consumption as a proxy for n-3 PUFAs is associated with a reduced risk of impaired cognitive functions (16 – 18). In the elderly and patients with AD, low DHA and n-3 PUFA levels have been detected in the plasma (19) and brain (20), showing widespread loss of neuronal synapses. Dietary DHA has been suggested to improve neuronal development (21), restore and enhance cognitive functions (22 – 24), and increase neuronal resistance to various types of insults, including amyloid-induced oxidative stress (25 – 28). To clarify the mechanisms of the beneficial effects of DHA on AD, the roles of DHA in cognitive functions and AD pathology are reviewed here.

2. Cognitive ability and DHA deficiency in the brain

DHA, one of the main structural lipids in the mammalian brain, is concentrated in membrane phospholipids at synapses and other neuronal membranes. It is critical for cellular functioning, normal brain development, and memory and cognitive processes in animals and humans (29 – 32). In aged animals, memory impairment occurs because of reduced levels of cerebral DHA (33, 34). Moreover, loss of the brain DHA content in patients with AD is accompanied by loss of memory and learning (12). This content may be reduced by enhanced free radical-mediated lipid peroxidation (35), decreased ability of dietary fatty acids to cross the blood-brain barrier because of impaired transport function in aging (36), decreased dietary intake, undefined impediments to the

uptake and utilization of n-3 PUFAs, or impeded shuttling of DHA from the liver to the brain (37). Further, a decrease in the level of serum DHA correlates with cognitive impairment (38). Escape latency is longer in n-3 PUFA-deficient animals than in control rats, suggesting that an n-3 PUFA-deficient diet may affect the process of habituation, a simple form of learning (39). DHA may also play a key role in the functioning of brain regions involved in the formation of new memories. Its deficiency affects mostly the cortex and hippocampus, areas that mediate learning and memory.

Epidemiological studies have suggested the neuroprotective consequences of diets enriched with n-3 PUFA (10, 15). Previously, we investigated whether dietary administration of DHA affects the memory-related learning ability of DHA-deficient rats (23, 24). Young and aged male DHA-deficient rats show considerably improved learning ability after intragastric administration of DHA. The beneficial effects were related to increases in the DHA level and DHA/arachidonic acid (AA) ratio in the cortico-hippocampal tissues, indicating that the hippocampal DHA level is directly associated with its dietary intake and that higher levels enhance hippocampus-dependent learning processes.

3. DHA and oxidative stress in the brain

Oxidative stress results from an imbalance between the formation and the degradation of pro-oxidants or impaired cellular antioxidant mechanisms, and excessive oxidative stress leads to cell damage and apoptosis (40). The brain is particularly susceptible to oxidative stress, because it has a high content of easily peroxidizable long-chain PUFAs such as DHA and AA, and mitochondrial consumption of a large quantity of glucose to fuel the brain's normal energy requirements results in relatively high production of free radicals (41).

In AD, the accumulation of amyloid-beta ($A\beta$) increases the production of free radicals, resulting in increased lipid peroxidation in the brain (41). Oxidative damage and formation of oxidized lipids and proteins have been observed in the brains of patients with AD during postmortem analysis (42). Crucial oxidative damage has also been observed in subjects with mild cognitive impairment (MCI), suggesting an early role of oxidative stress (43). Lipid peroxide (LPO) levels are significantly lower in DHA-administered rats and reciprocally correlate with the DHA/AA ratio (26, 27, 44), indicating that dietary DHA contributes to the antioxidant defense, decreases oxidative stress, and protects against memory loss. This inference is consistent with the fact that DHA also increases the levels of antioxidant enzymes such as catalase and glutathione peroxidase and reduces

glutathione levels with a concomitant decrease in the levels of reactive oxygen species (ROS) in the cortex and hippocampus of aged and AD model rats (26, 27). In an in vitro cell model, however, DHA did not prevent the oxidative stress induced in neurons exposed to an A β peptide solution (45, 46).

Further, DHA has free radical-scavenging properties such as protection against lipid and protein peroxidation in developing and adult brains and attenuation of neuronal loss and cognitive and locomotor deficits in animal models of ischemia–reperfusion brain injury (47–49). The antioxidant action of DHA in the brain has been underscored (50), despite its molecular structure containing six double bonds, which theoretically makes it a molecular target for peroxidation and sensitizes cells to ROS. Moreover, DHA can produce docosatrienes and resolvins, collectively known as docosanoids. In particular, neuroprotectin D1 [(10,17*S*)-dihydroxydocosatriene, NPD1] is formed through tandem phospholipase A₂–lipoxygenase action on free DHA; it is a docosatriene that appears to be a major bioactive effector in neuronal tissues. Even a minute amount (in the picogram to nanogram range) of NPD1 can promote anti-inflammatory and neuroprotective activity and inhibit oxidative stress-induced apoptosis (51). These results suggest the notable role of DHA and/or DHA-derived mediators in the inhibition of oxidative stress.

4. DHA and AD

4.1. Human studies

AD is a neurodegenerative disease that commonly affects the elderly and is characterized by loss of short-term memory and cognitive impairment. Its cause is unknown; however, the disease is associated with major pathological traits such as overproduction and accumulation of A β produced by the proteolytic processing of amyloid precursor protein (APP) (52), formation of neurofibrillary tangles by the aggregation of Tau protein within neurons (53), and neuronal loss. Although there is currently no treatment for AD, there is growing interest in the role of diet for its prevention and treatment.

Numerous epidemiological studies have shown an inverse association between AD risk and n-3 PUFA dietary intake. A population-based prospective study in Rotterdam, The Netherlands, showed that consumption of fish, an important source of n-3 PUFAs, is inversely related to dementia, particularly AD (15). In a prospective human study on the progression of AD, the total intake of n-3 PUFAs, particularly DHA, but not EPA, was associated with reduced risk of AD (10). Moreover, cross-sectional analyses have linked low levels of plasma DHA with dementia and with AD in particular (54). Moreover, pa-

tients with blood DHA concentrations in the highest quartile demonstrated lower risk of developing dementia than those with blood DHA concentration in the lowest three quartiles, during a mean follow-up of 9 years (55). We investigated the relationships among cognitive function, nutrition, and fatty acid components of plasma and erythrocytes in 53 community dwellers (≥ 65 years) in Shimane, Japan, over a 4-year follow-up period: the DHA levels in erythrocytes were significantly higher in those who showed improvement or no change in their Hasegawa dementia rating scale (HDS-R) score than in those who showed a worse score (Table 2) (unpublished data). This suggests that even in elderly Japanese who consume more fish than elderly people in the West, more consumption of dietary DHA may protect against the age-related decline of cognitive function observed in the elderly with very mild dementia. Taken together, these results suggest that dietary supplementation with DHA alters the risk of cognitive impairment with aging and/or developing AD over the long term and that low blood concentration of DHA are a critical risk factor for cognitive dysfunction.

Despite the promising findings of epidemiological studies, no effect of EPA or DHA on AD has been observed in clinical studies (56–58). In a well-designed, randomized, double-blind, placebo-controlled trial of n-3 PUFA supplementation, no significant differences in the primary outcome, measured by Mini-Mental State Examination (MMSE) and AD Assessment Scale cognitive component (ADAS-cog), were observed between the treated and the placebo groups; however, a subgroup analysis of mildly affected individuals (MMSE score

Table 2. Erythrocyte fatty acid profiles of community-dwelling elderly people followed up for 4 years; this data was obtained as part of the Nutrition-Cognition Study in Shimane, Japan (unpublished data)

	Improved or unchanged group (n = 40)	Aggravated group (n = 13)
EPA (mol%)	2.54 \pm 0.77	2.52 \pm 0.87
DHA (mol%)	9.98 \pm 1.07	9.09 \pm 1.25*
EPA/AA	0.26 \pm 0.09	0.27 \pm 0.11

Values indicate means \pm S.E.M. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid. Elderly people were divided into two groups based on changes in the Hasegawa dementia rating scale (HDS-R) at follow-up after 4 years: an improved or unchanged group (–1 point to +6 points, 75.3 years old) and an aggravated group (–2 or more point decrease, 76.6 years old). Erythrocyte fatty acids were measured by gas chromatography. * $P < 0.05$. This cohort study was performed by the collaboration between Michio Hashimoto (Shimane University Faculty of Medicine, Izumo, Japan), Kazuya Yamashita (Shimane Nursing College, Izumo, Japan), and Setsushi Kato (Jinjuikai Kato Hospital, Kawamoto, Japan).

> 27) demonstrated a significant reduction in MMSE-assessed decline compared with a placebo subgroup (56). The authors concluded that the beneficial effects of n-3 PUFA supplementation may depend on the cognitive status at the start of treatment, with those in the earliest stages of the disease benefiting the most. The contrasting findings of the aforementioned epidemiological and clinical studies suggest that n-3 PUFAs may be effective only when consumed before the onset of the disease or when symptoms are mild.

4.2. Studies in AD-model animals

Given that AD is pathologically characterized by inter-neuronal and intraneuronal deposition of A β peptides (59), the direct infusion of these peptides into the brain has helped to produce AD-model animals (60, 61). The interventions involving dietary DHA have created opportunities for replicating the epidemiological claims regarding the role of DHA in delaying or possibly preventing the development of AD. For example, AD-model rats produced by intraventricular infusion of A $\beta_{(1-40)}$ peptide solution have been used to evaluate the effects of dietary DHA administration on their learning ability through shuttle-box avoidance situations (26) and eight-arm radial maze tasks (27); the results are summarized in Table 3. Altogether, these results suggest protective and ameliorative effects of dietary DHA on the impairment of learning ability in AD-model rats. Further, DHA supplementation has been shown to improve cognitive function, as measured with the Morris water maze, even late in the life of transgenic APP harboring the Swedish mutation (APP^{swe}) (Tg2576) AD-model mice, (28).

Therefore, DHA is a possible therapeutic agent for protection against AD and amelioration of the learning deficiencies attributed to this disease.

5. Mechanisms of the preventive effects of DHA in AD-model rats

5.1. DHA and its incorporation into the brain

DHA-administered vehicle and AD-model rats have high plasma DHA levels, suggesting effective intestinal absorption of orally administered DHA (26, 27). The administration of DHA substantially increased the DHA level in the hippocampus and concomitantly elevated the DHA/AA molar ratio in both DHA-administered vehicle and DHA-administered AD-model rats compared with vehicle and AD-model groups without DHA administration. Further, significant positive correlations were observed between the cerebral cortex and the plasma DHA levels as well as between the hippocampal and the plasma DHA levels of all analyzed rats (27), suggesting the substantial penetration of DHA into the cortico-hippocampal regions of the brain.

In the *de novo* system, each PUFA is possibly metabolized after being taken into cerebral endothelial cells and astrocytes, which constitute the blood–brain barrier and then released from both types of cells. Further, DHA, as the metabolite, is absorbed from the extracellular medium by neurons after its release from glial cells or the capillary endothelium (3). In addition, the synaptosomal plasma membrane fluidity of the cerebral cortex has been found to decrease in A β -induced AD-model rats; this decreased fluidity was prevented by the pre-administra-

Table 3. Effect of dietary pre- or post-administration of DHA on the memory-related learning ability and oxidative stress in the amyloid β_{1-40} -infused Alzheimer's disease (AD)-model rats

	A β_{1-40} (AD-model rats)	DHA + A β_{1-40} (pre-administration)	A β_{1-40} + DHA (post-administration)
Memory-related learning ability			
1) Avoidance task			
Active avoidance responses	↓	↑	↑
2) Radial maze task			
Reference memory errors	↑	↓	↓
Working memory errors	↑	↓	↓
Oxidative stress			
1) Lipid peroxide	↑	↓	↓
2) Reactive oxygen species	↑	↓	↓
3) HADF	↑	↓	N.D.

A β_{1-40} , amyloid β_{1-40} peptide; DHA, docosahexaenoic acid; HADF, apoptosis-related histone-associated DNA fragments; N.D., not detected. The AD-model rats were prepared by the infusion of A β_{1-40} into the cerebral ventricle of rats. This table was constructed from the data of Refs. 26 and 27.

tion of DHA (62).

The infusion of $A\beta$ into the rat hippocampus evidently induces deficits in long-term potentiation (LTP) and working memory (63). Moreover, acetylcholine levels in the brains of $A\beta$ -infused rats decrease similarly to those in patients with AD experiencing memory impairment (60). Dietary supplementation with DHA restores neurotransmitter release and reverses the deficits in LTP (64). DHA is crucial for the induction of LTP, and when released endogenously during stimulation, it is sufficient to trigger LTP (65). Dietary DHA increases cortical acetylcholine levels and concurrently improves avoidance performance (66). Learning and memory depend on the dendritic-spine activity of neurons, and DHA increases the density of dendritic spines (28). These findings indicate that DHA-induced alterations in the synaptic plasma membrane fluidity may contribute to the synaptic plasma membrane-related functions that constitute learning and memory in AD-model rats.

5.2. Oxidative stress, apoptosis, and DHA

Oxidative stress is closely related to the pathogenesis of AD because it occurs as an early event in the progression of AD and before the development of senile plaques and neurofibrillar tangles (67). $A\beta$ deposition and hyperphosphorylated Tau are believed to function as compensatory responses and downstream adaptation to avert oxidative stress-induced death (68). Therefore, vitamin E and other antioxidant agents have been widely used in clinical trials undertaken to demonstrate their beneficial effects, although results have often been controversial (69). Nonetheless, the inconsistencies may be explained on the basis of a recent study in a young transgenic AD mouse model, which showed that antioxidant treatment may actually be helpful only in the early stages of the disease (70).

Oxidative stress increases enormously in the brains of AD rats, although the cause-effect relationship is yet to be established (59). The levels of LPO and ROS in the cerebral cortex and hippocampus increase significantly after the infusion of $A\beta$ into the cerebral ventricle of rats, but then decrease significantly after the dietary administration of DHA (Table 3). NPD1, a DHA-derived mediator, also possesses neuroprotective properties and inhibits oxidative stress. Considering that DHA (10, 12, 71) and NPD1 (51) levels are low in the hippocampus of patients with AD, adequate intake of DHA to ensure sufficient levels for conversion to NPD1 may prevent neuronal oxidative stress related to the pathogenesis of AD. These data suggest that dietary DHA increases the antioxidant defense in the brain and the mechanism may be related to the induction of antioxidant enzymes (26, 44).

Both biochemical and immunohistochemical studies

have suggested that $A\beta$ -induced apoptosis, or neuronal death, is a characteristic of $A\beta$ -infused AD-model rats (60, 61). The comparatively lower concentration of cytoplasmic DNA fragments in the cortex and hippocampus of DHA-pre-administered $A\beta$ -infused AD-model rats could therefore be ascribed to the defensive action of DHA against apoptosis-like neuronal death (Table 3) because these fragments increase with apoptotic cell death. DHA and NPD1 decrease $A\beta_{1-42}$ -induced apoptosis of human neurons and glia by up-regulating anti-apoptotic genes encoding Bcl-2, Bcl-xl, and Bfl-1 (51). This effect is associated with the formation of NPD1. Moreover, DHA modulation of the phosphatidylinositol-3-kinase (PI3K/Akt) pathway, critical for cell survival, is considered another mechanism through which DHA exerts its anti-apoptotic effect on neurons (72). All these findings suggest that DHA prevents the reduction of memory function in AD by preventing $A\beta$ -induced neurodegeneration, although the mechanism by which it does so is yet to be clarified.

5.3. Glucose transport and DHA

Rats deficient in n-3 PUFAs have decreased brain glucose utilization associated with reduced glucose transporter-1 (GLUT1) immunostaining and protein expression of endothelial and astrocytic GLUT1 (73, 74). Exposure of primary rat brain endothelial cells (RBECS) to DHA, but not AA, increases the uptake of glucose (75), and DHA supplementation to the cells increases basal glucose transport in RBECS, associated with increased GLUT1 protein levels (76). Changes in GLUT1 activity could result from variations in membrane structure and physical properties (fluidity) subsequent to the incorporation of DHA into membrane phospholipids (62, 77), assuming that changes in GLUT1 conformation and its affinity for glucose alter glucose uptake into cerebrovascular endothelial cells. During healthy aging, brain glucose uptake decreases significantly in specific cortical regions (78), and this effect is more pronounced in the elderly with deteriorating cognitive function, as in AD (79). These data suggest the possible role of DHA in the control of GLUT1 expression and glucose transport to the brain.

5.4. Anti-inflammatory effects and DHA

Dietary DHA supplementation results in a low AA content in the hippocampus and cortex of $A\beta$ -infused AD-model rats (26, 27) and neuronal membrane phospholipids of double and triple transgenic mice (80, 81), in association with cognitive preservation. Although much is known about the role of AA in the generation of eicosanoids by cyclooxygenases, including prostaglandins, and their role in initiating and maintaining the in-

inflammatory cascade in the brain, the critical roles of DHA and other n-3 PUFAs are beginning to be understood. Analogous to the formation of eicosanoids derived from AA, DHA is modified through phospholipase A₂ and lipoxygenase to form NPD1 (51, 82, 83). Several studies have demonstrated that DHA reduces the activity of phospholipase A₂ in the brains of patients with AD (84, 85). Thus, DHA-based neuroprotection may involve anti-inflammatory processes that reduce AA availability. Although contradictory data have been published in epidemiological and clinical studies regarding nonsteroidal anti-inflammatory drugs in the prevention and treatment of AD, DHA together with aspirin has been proposed as a strategy to explore its potential for preventing neurodegenerative diseases such as AD (86). Further knowledge of the molecular mechanisms through which increases in the levels of NPD1 and other metabolites of DHA enhance neuronal health is required to understand how decreased levels of these important metabolites might contribute to the pathogenesis of AD and other degenerative diseases.

5.5. Lipid rafts and DHA

Lipid rafts are lipid microdomains in cell plasma membranes (87) that function as organizational sites involved in compartmentalization, protein transfer, proteolytic processing, and formation of cell signaling complexes (88). In particular, low-density detergent-insoluble membrane domains are the structural and functional floating units that may be involved in numerous signal transductions (89). In intact cells, they appear as flask-shaped concave structures, remain invaginated in the plasma membrane, and act as platforms for the generation and/or transmission of signals associated with amyloid peptides (90) and amyloidogenesis. In vitro studies of human neuroblastoma cells have also shown that lipid rafts are highly stable structures, rich in cholesterol and glycosphingolipids, and play a role in signal transduction cascades (91). Amyloidogenesis involves interactions among amyloid peptides, cholesterol, saturated fatty acids, and sphingomyelin (92). Administration of DHA considerably decreases the levels of cholesterol, palmitic acid, and stearic acid in detergent-insoluble membrane fractions of AD-model rats (93), suggesting raft-destabilizing effects of DHA, which might induce the collapse of rafts and decrease the docking of amyloids onto raft platforms. DHA-induced increases in membrane fluidity (61, 92, 94) may help in exocytosis to relieve the amyloid burden. In the biochemical process of amyloidogenesis, membrane-soluble APP of neuronal plasma membranes is cleaved by sequential enzymatic actions of γ - and β -secretase into small fragments of A β _{1–40} and/or A β _{1–42} amino acid sequences. Amyloid peptides then precipitate,

after conformational transformation, into insoluble proteic deposits as neuritic plaques and neurofibrillary tangles. DHA decreases amyloid levels in detergent-insoluble membrane fractions from the cortex of AD-model rats (93). Alterations in lipid raft and membrane composition, as well as in the fluidity of the membrane in synaptosomal preparations, have been well characterized in AD-model rats (62, 93). Consequently, we tested whether DHA inhibits the in vitro fibrillation of A β peptides.

6. In vitro amyloid fibrillation and DHA

DHA directly inhibits fibrillation and formation of toxic A β in vitro. Thioflavin T (ThT) has the intrinsic property of binding with β -sheeted amyloids but not with the monomeric A β forms (95). DHA-incubated amyloid samples demonstrate low ThT-fluorescence intensity, indicating low fibrillation (96–98). DHA also significantly affects the morphology of amyloid fibrils viewed by transmission electron microscopy; the presence of fibers is very low in the presence of DHA (Fig. 1: A and B). A magnified view indicates intertwined appearances of A β _{1–40} fibrils (Fig. 1C). In the presence of DHA, the extent of fibrillation and morphology of fibrils are markedly affected and demonstrate an amorphous conformation (Fig. 1D). These results indicate that DHA inhibits the extent of A β fibrillation.

Amyloid oligomers, more so than mature fibrils, have been attributed to the pathology of AD (64, 65). Clinical studies have shown that soluble A β levels have a greater correlation with dementia severity than amyloid deposits (99). Furthermore, in the brain of patients with AD, A β oligomers mainly target synapses affected early in the pathogenesis of the disease (100). Cognitive deficits appear before amyloid deposition in transgenic AD-model mice (101). These findings suggest that the very early stages of AD (MCI) may be due to synaptic dysfunction caused by A β oligomers. In contrast, DHA stabilizes A β oligomers and sustains their induced toxicity in PC12 cells (102). This stabilizing effect is, however, not observed on A β _{1–42} harboring Arctic mutation E22G, which provides new insights into how endogenous lipids can affect the toxicity of different A β aggregates.

Previously, we have shown that DHA-induced inhibition of fibrillation occurs at oligomer levels with a molecular mass of approximately 15 kDa and is dose dependent, suggesting that the DHA-induced inhibition of oligomer formation is one of the mechanisms by which DHA constrains in vitro fibrillation (96, 97). The mechanism through which DHA inhibits the oligomerization of A β remains to be explored. Amyloid deposition proceeds in an orderly manner to form insoluble amyloid fibrils from the soluble monomeric form. The generally held

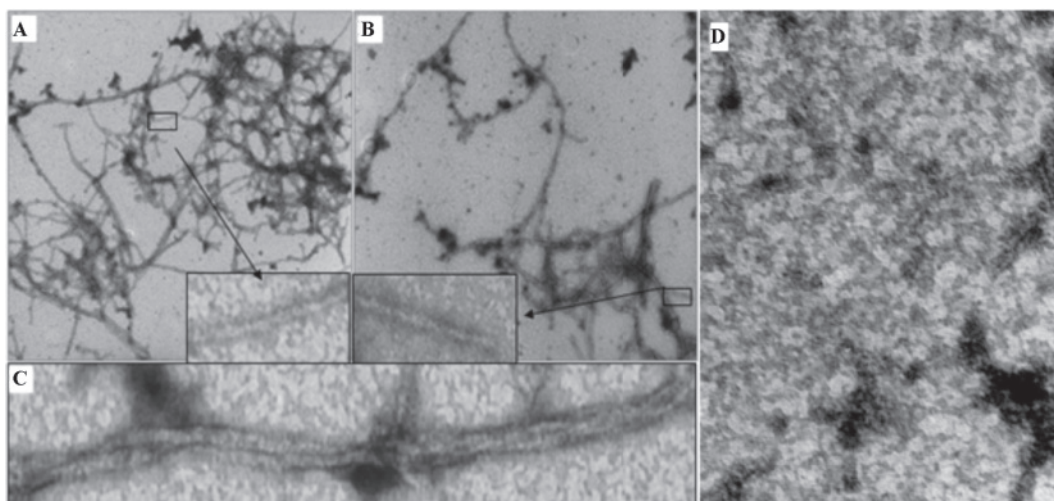


Fig. 1. The effect of docosahexaenoic acid (DHA) on amyloid-beta ($A\beta$) fibril formation. $A\beta_{1-40}$ was incubated with (B) or without (A) DHA (20 μ M) for 24 h. Then, 4 μ l of each sample was subjected to a 400-mesh grid, dried for 1 min, stained with 1% uranyl acetate, and visualized under an electron microscope. The fibrils appeared as both single (Inset of A and B) and intertwined (C) strands. Most of the grids of DHA-treated samples showed an amorphous consistency (D).

view (103) is that helical monomeric units are misfolded into β -sheets that run orthogonally to produce the so-called prefibrillar seeds or oligomers (protofilaments); protofilaments then elongate, through the stacking of more β -sheet units, to rod-like structures, which finally mature into fibrils by further extension. The driving force for this phenomenon involves both hydrophobic and electrostatic interactions (104). DHA has its own high hydrophobic volume.

The association of C-terminal amino acid R-groups with the central hydrophobic core of $A\beta$ peptides is a crucial determinant of amyloid polymerization (97, 105), and the rapid fibrillation rate of $A\beta_{1-42}$ is attributed to increased hydrophobicity (97, 106). The presence of aromatic residues, particularly of tyrosine and phenylalanine, plays a major role in molecular recognition, promotes amyloid formation, and stabilizes the resulting fibrils (107). The underlying mechanism includes lateral stacking of the aromatic rings of β -sheets (108). The fact that tyrosine 10 (Tyr10) in the $A\beta_{1-42}$ backbone engrosses a region that acts as a site for intermolecular stacking during fibrillation is based on the observation that Tyr10-intrinsic emission of $A\beta_{1-42}$ monomers decreases during fibrillation and increases during defibrillation (97). The DHA-induced inhibition of $A\beta_{1-42}$ fibrillation reduces Tyr10 emission, demonstrating that the inhibition perturbs the local environment of Tyr10 (97). Tyr10 has notable implications in $A\beta$ fibrillation; in particular, the Tyr10-gated electron with concomitant formation of dityrosine is a key factor in the toxic mechanism of $A\beta$ (109). In a previous study, the levels of dityrosine increased in the $A\beta_{1-42}$ control, and DHA reduced the levels

of dityrosine, indicating that DHA-induced inhibition of $A\beta_{1-42}$ fibrillation is, at least partially, related to its anti-dityrosine property (97). In addition, DHA has a strong anti-order property because of its inherently large hydrophobic volume, higher average area per molecule, and spring-like efficacy (110). In contrast, $A\beta$ fibers are sterically stacked and highly ordered structures. The hydrophobic properties of DHA may therefore be related to its anti-fibril properties. Nevertheless, further studies are needed to clarify the mode of DHA actions on the inhibition of in vitro fibrillation.

The key question is whether the DHA-induced inhibition of $A\beta$ fibrillation also inhibits the $A\beta$ -induced toxicity in vitro. The redox level of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), a cytotoxicity marker to measure cell viability, is lower in $A\beta_{1-42}$ -incubated neurons than in $A\beta_{1-42}$ + DHA-incubated neurons, indicating that DHA considerably inhibits $A\beta_{1-42}$ -induced toxicity in neurons (97). Notably, DHA alone increases the redox activity as compared with that of untreated (vehicle) cells, indicating that the higher redox activity (toxicity-inhibitory potential) is ascribable to DHA. Morphologically, $A\beta_{1-42}$ causes severe axodendritic loss; in culture, floating debris is also higher in these cells, suggesting degeneration. In contrast, the DHA-incubated cells have higher dendro-axonal appearances with a healthier morphology (Fig. 2). Taken together, these observations demonstrate that DHA inhibits in vitro $A\beta$ fibrillation and in so doing, reduces the $A\beta$ oligomer-induced toxicity in neurons.

A question may arise about whether the effects of DHA in vitro have an impact on the protection from AD

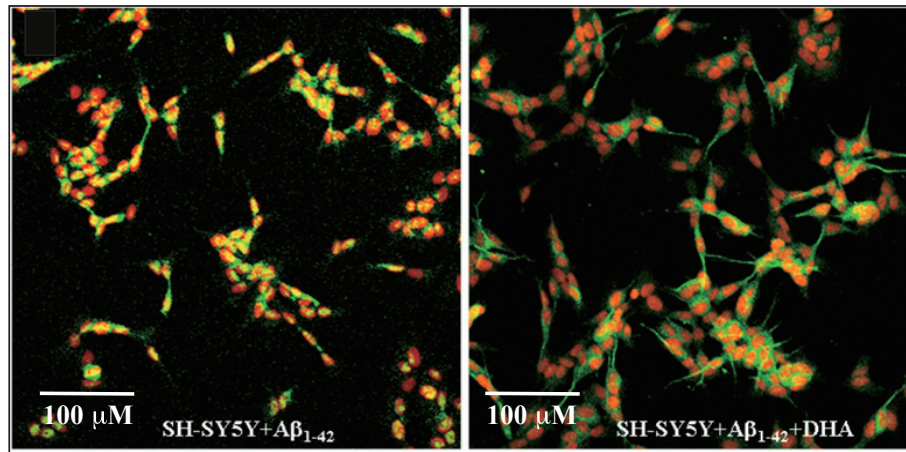


Fig. 2. The effect of decosahexaenoic acid (DHA) on amyloid-beta ($A\beta$)-induced toxicity in neurons. Neuronally differentiated SH-SY5Y cells were incubated with $A\beta_{1-42}$ in the presence or absence of DHA for 24 h to commence fibrillation in the medium. The qualitative effect of DHA is shown by the representative morphological changes in the cells treated with $A\beta_{1-42}$ alone (left panel) and with $A\beta_{1-42}$ plus DHA (right panel). Altered neuritic sprouting with dystrophic axodendritic systems was clearly observed after the treatment of cells with $A\beta_{1-42}$ for 24 h. However, DHA inhibited the toxicity, as indicated by the appearance of well-defined axodendritic sprouting processes, good cell viability, and full spherical somas. Adapted with permission from Ref. 97.

because the concentration of free DHA in the brain is very low. In vivo studies demonstrated that oral administration of DHA decreases the amyloid burden in the cortico-hippocampal tissues of $A\beta$ -infused AD-model rats (101) and in the hippocampus of APPsw (Tg2576) transgenic AD-model mice (30), in association with an increase in DHA levels of these brain tissues by 20% – 30%. The net increase in the cortico-hippocampal tissues of AD-model rats is 19 – 50 nmol/mg protein, comparable to the 50 nmol unesterified DHA/mg protein content measured in the human hippocampus (51). In vitro, 5 – 20 μ M DHA inhibits $A\beta$ fibrillation, and 20 μ M could be considered the best concentration to demonstrate the anti-amyloid properties of DHA (96 – 98). On average, this is equivalent to 2.5:1 (mol:mol ratio) of $A\beta$ versus DHA. Therefore, one may hypothesize that DHA inhibits the elongation of fibers and/or causes dissolution of mature fibers by intercalating between $A\beta$ sheets (97, 98).

The concentration of free DHA commonly present in the brain or cerebrospinal fluid (CSF) is unclear. The concentration of DHA in human CSF is about 0.2 μ M (111), which is in the range (0.5 μ M) used to inhibit the amyloid-induced toxicity in SH-SY5Y experiments in vitro (98). Therefore, DHA at 0.5 μ M could be presumed to act under physiological conditions. It did not, however, significantly affect in vitro $A\beta$ fibrillation, and 10 – 20 μ M of DHA was required for significant inhibition (97). This may be related to the differences in the in vivo and in vitro cell culture conditions and those of the environments.

7. Neurogenesis and DHA

During the last several years, new experimental data have evidenced that the brain can create new neurons. Considering that the hippocampus is a focal point for the formation and retrieval of memories, many researchers believe that new neurons may be essential for learning and memory (112) and can be generated from stem cells in the dentate gyrus (DG) of the hippocampus throughout life (113, 114); in the DG, newborn neurons extend axonal projections to the hippocampal CA3 regions, which is the basis of associative learning, and hippocampal neurogenesis correlates with the performance of memory tasks (115, 116). These findings indicate that adult hippocampal neurogenesis is associated with the formation and function of memory and learning ability. With this in mind, DHA could be assumed to play crucial roles in the development and functions of brain neurons.

The effect of DHA on neuronal differentiation of neural stem cells in vitro and in vivo has been assessed (117): in the presence of DHA, neuronal stem cells obtained from 15.5-day-old rat embryos substantially increased the number of Tuj1 (a neural marker)-positive neurons compared with the control on two culture days, and the newborn neurons in the DHA group were morphologically more mature than those in the control. In vivo, chronic administration of DHA for 12 weeks significantly increased the number of 5-bromo-2'-deoxyuridine(+)/NeuN(+) newborn neurons in the granule cell layer of the DG of adult rats (117). These results demonstrate that DHA effectively promotes neurogenesis

both in vitro and in vivo and suggest that DHA modulates hippocampal function regulated by neurogenesis, neuronal growth, dendritic arborization, and synaptogenesis and thereby has cognition-enhancing ability. The role of DHA in this process is modulated through the expression of basic helix-loop-helix transcription factors in neuronal stem cells originating in the DG (118).

Considering that n-3 PUFAs have trophic neural effects, higher consumption of these fatty acids is expected to correlate positively with the volume of gray matter in regions of the brain such as the amygdala, hippocampus, and anterior cingulate cortex. These regions are core components of the distributed corticolimbic circuitry that regulates emotional arousal in response to the regulation of affect in the service of adaptive behavioral responses. Higher consumption of long-chain omega-3 fatty acids is associated with a larger volume of gray matter in the nodes of the corticolimbic circuitry supporting emotional arousal and regulation (119). DHA promotes substantial changes in neurotransmitter function, brain structure, and

behavior. For example, animals fed DHA-deficient diets show reduced frontal cortex concentrations of monoamine neurotransmitters such as dopamine and serotonin (120). The number of Fos-positive neurons in the CA1 region of the hippocampus increases significantly in rats administered DHA, with a significant negative correlation between the number of Fos-positive neurons and the reference memory errors measured by tasks in an eight-arm radial maze (121). The Fos protein, encoded by the immediately early gene *c-fos*, is a transcription factor and functional marker of neuronal activity. Thus, DHA intake could influence brain morphology through some or all of these mechanisms. Such associations may mediate the previously observed effects of n-3 PUFAs on memory, mood, and affect regulation.

8. Conclusion

In this review, we have summarized the findings of previous in vivo and in vitro studies and have suggested

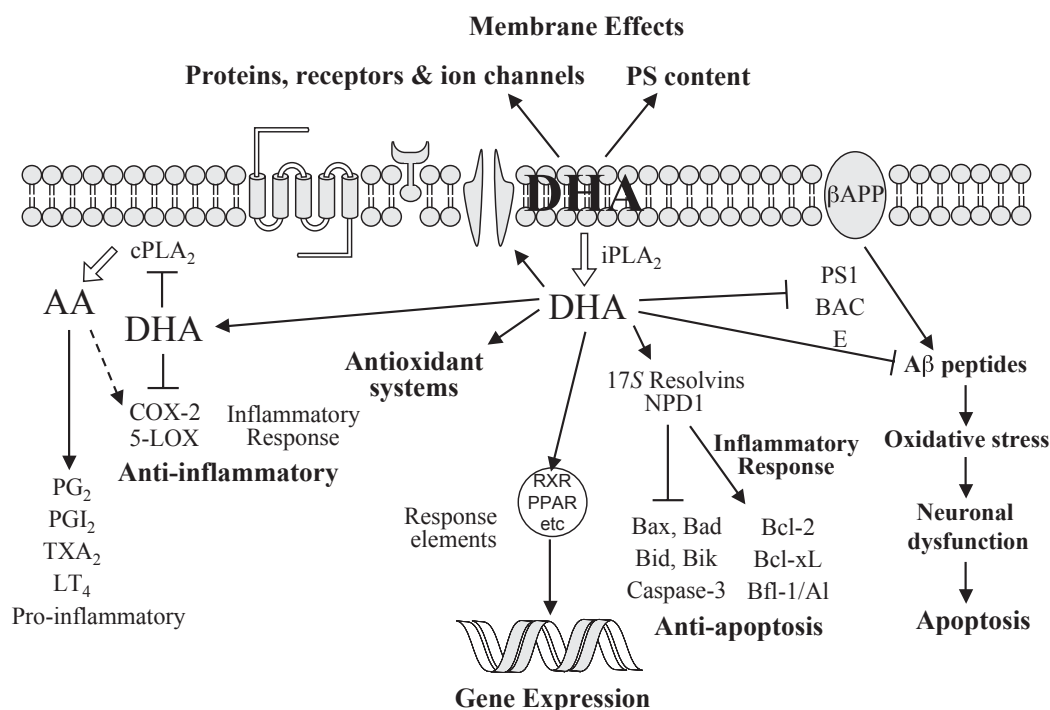


Fig. 3. The likely molecular mechanisms underlying the neuroprotective effects of DHA. These effects are related to direct actions on plasma membranes, inflammatory responses, and gene expression; refer to the text for a detailed explanation of the mechanisms. The membrane effects appear to be related to alterations in the biophysical properties of the cell membranes and modulation of phosphatidylserine. Further, nonesterified DHA is the precursor of anti-inflammatory resolvins such as the neuroprotectin D1 (NPD1), which inhibits pro-apoptotic proteins and enhances apoptotic ones. Nonesterified DHA also regulates gene expression, influences ion channels, and enhances endogenous antioxidant systems. Moreover, it suppresses amyloidogenesis by inhibiting the generation of Aβ peptides. In the figure, solid arrows indicate positive effects, arrowheads indicate inhibition, dotted arrows indicate competition, and open arrows indicate phospholipase A₂-induced release from cell membranes. Abbreviations: COX-2, cyclooxygenase-2; BACE, beta-site APP cleaving enzyme; βAPP, amyloid precursor protein beta; cPLA₂, cytosolic PLA₂; iPLA₂, Ca²⁺-independent PLA₂; 5-LOX, 5-lipoxygenase; LT, leukotriene; PG, prostaglandin; PGI, prostacyclin; PPAR, peroxisomal proliferator-activated receptor; PS phosphatidylserine; PS1, presenilin 1; RXR, retinoid X receptor; TXA, thromboxane.

that dietary administration of DHA increases neuronal cell functions, by its incorporation into neural membrane phospholipids. DHA thereby prevents neuronal damage and death in vitro and protects the elderly as well as animal models from age- and $A\beta$ -induced cognitive impairments in vivo.

DHA protects against memory impairment of $A\beta$ -infused AD-model rats, and the protective effect is accompanied by increased DHA content in membrane phospholipids of the cortico-hippocampal regions. DHA is possibly absorbed from the extracellular medium by neurons after its release from astrocytes or cerebral endothelial cells, which constitute the blood-brain barrier (3). This uptake may correlate with the inhibition of amyloid polymerization and subsequent enhancement of memory-related learning ability. Although the mechanisms of the beneficial effects of DHA for the prevention of AD are not yet clear, increasing levels of this PUFA in the cortico-hippocampal regions of aged and AD-model rats have been shown to result in neuroprotection in these regions. The molecular mechanisms of the neuroprotective effects of DHA are depicted in Fig. 3.

Recent studies have indicated the apparently crucial role of DHA in preventing AD and its very mild, precocious stages. Studies on the exact molecular mechanisms underlying the beneficial effects of DHA are required to validate the hypothesis that changing dietary habits or promoting dietary supplementation of DHA can considerably improve human health and, especially, prevent or delay cognitive impairment in mild cases of AD. Further effort to accumulate knowledge in this field of research is needed now.

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