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Membrane lipid modifications and therapeutic effects mediated by hydroxydocosahexaenoic acid on Alzheimer's disease ☆

Manuel Torres [a,*], Samantha L. Price [b], Maria A. Fiol-deRoque [a], Amaia Marcilla-Etxenike [a], Hasna Ahyayauch [c], Gwendolyn Barceló-Coblijn [a], Silvia Terés [a], Loukia Katsouri [b], Margarita Ordinas [a], David J. López [a], Maitane Ibarguren [a], Félix M. Goñi [c], Xavier Busquets [a], Javier Vitorica [d], Magdalena Sastre [b,**], Pablo V. Escribá [a,*]

[a] Laboratory of Molecular Cell Biomedicine, University of the Balearic Islands, Palma de Mallorca, Spain
[b] Division of Brain Sciences, Imperial College London, London, United Kingdom
[c] Biophysics Unit (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Bilbao, Spain
[d] IBIS Seville Biomedical Research Institute, Virgen del Rocio University Hospital, CSIC — University of Seville, and CIBERNED, Seville, Spain

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative pathology with relevant unmet therapeutic needs. Both natural aging and AD have been associated with a significant decline in the omega-3 polyunsaturated fatty acid docosahexaenoic acid (DHA), and accordingly, administration of DHA has been proposed as a possible treatment for this pathology. However, recent clinical trials in mild-to-moderately affected patients have been inconclusive regarding the real efficacy of DHA in halting this disease. Here, we show that the novel hydroxyl-derivative of DHA (2-hydroxydocosahexaenoic acid — OHDHA) has a strong therapeutic potential to treat AD. We demonstrate that OHDHA administration increases DHA levels in the brain of a transgenic mouse model of AD (5xFAD), as well as those of phosphatidylethanolamine (PE) species that carry long polyunsaturated fatty acids (PUFAs). In 5xFAD mice, administration of OHDHA induced lipid modifications that were paralleled with a reduction in amyloid-β (Aβ) accumulation and full recovery of cognitive scores. OHDHA administration also reduced Aβ levels in cellular models of AD, in association with alterations in the subcellular distribution of secretases and reduced Aβ-induced tau protein phosphorylation as well. Furthermore, OHDHA enhanced the survival of neuron-like differentiated cells exposed to different insults, such as oligomeric Aβ and NMDA-mediated neurotoxicity. These results were supported by model membrane studies in which incorporation of OHDHA into lipid-raft-like vesicles was shown to reduce the binding affinity of oligomeric and fibrillar Aβ to membranes. Finally, the OHDHA concentrations used here did not produce relevant toxicity in zebrafish embryos in vivo. In conclusion, we demonstrate the pleitropic effects of OHDHA that might prove beneficial to treat AD, which suggests that an upstream event, probably the modulation of the membrane lipid composition and structure, influences cellular homeostasis reversing the neurodegenerative process. This Article is Part of a Special Issue Entitled: Membrane Structure and Function: Relevance in the Cell's Physiology, Pathology and Therapy.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder that produces severe cognitive impairment as it progresses. This pathology is the main neurological cause of dementia and it is suffered by 36 million people worldwide, elderly adults in most cases (World Alzheimer Report 2011). Unfortunately, there are still no effective treatments that mitigate the neurological deficits associated with AD. Currently, these patients may be treated with two classes of approved drugs that ameliorate the symptoms of AD, acetylcholinesterase inhibitors and NMDA receptor antagonists, although their clinical efficacy is considered to be very limited [1]. Other promising therapeutic approaches have been proposed for AD, such as statins and non-steroidal anti-inflammatory drugs, although they have yet to offer conclusive results in clinical trials [2–4].

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; PS1, presenilin-1; Aβ, amyloid-β peptide; GFP, green fluorescent protein; WT, wild type; LC/MS, liquid chromatography/mass spectrometry; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol; Cho, cholesterol; SM, sphingomyelin; PUFA(s), polyunsaturated fatty acid(s); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; OHDHA, 2-hydroxydocosahexaenoic acid

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* Corresponding authors at: Laboratory of Molecular Cell Biomedicine, Department of Biology, University of the Balearic Islands, Crta. Valldemossa km. 7.5, 07122 Palma de Mallorca, Spain. Tel.: +34 97117 3331; fax: +34 97117 3184.

** Correspondence to: Division of Brain Sciences, Imperial College London, Hammersmith Hospital, Du Cane Road, W12 0NN London, United Kingdom. Tel.: +44 2075946673; fax: +44 2075946548.

E-mail addresses: manuel.torres.phd@gmail.com (M. Torres), m.sastre@imperial.ac.uk (M. Sastre), pablo.escriba@uib.es (P.V. Escribá).

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DHA (22:6 n-3) is the most abundant omega-3 PUFA in the brain and it is tightly involved in the functioning of the central nervous system (CNS) [5], particularly in neurogenesis, synaptogenesis and synaptic transmission [6,7]. This fatty acid is obtained through the diet and its deficiency is associated with age-related cognitive decline and with neurodegenerative diseases, such as AD [8,9]. In recent years, PUFAs like DHA have gained much attention due to promising results that suggest they may be useful to treat AD. In this sense, several studies have demonstrated that oral intake of DHA or fish oil reduces AD-associated brain pathology, for instance, improving cognitive deficits, protecting against synaptic degeneration and lowering Aβ levels in transgenic AD mouse models [10–13]. Moreover, these results are supported by epidemiological studies indicating an inverse relationship between DHA intake and AD incidence, which correlate high DHA levels with reduced risk of cognitive dysfunction [14,15]. However, direct administration of DHA in clinical trials only showed improved cognition of a small subgroup of patients with very mild cognitive dysfunction and there was no clear effect in most patients [16,17], even though DHA administration improves the physiological, but not pathological, age-related cognitive decline [18].

In this context, there would appear to be a link between AD and lipid alterations in neuronal membranes, especially diminished DHA levels. Therefore, molecules that are effective in restoring DHA and normalizing the membrane lipid composition could constitute therapeutic tools to treat AD. In the present work, we show that OHDHA regulates membrane lipid composition and structure, cell signaling and, additionally, it improves cognitive scores in animal models of AD [19], thereby representing a novel therapeutic candidate for the treatment of AD. This DHA derivative bears a hydroxyl group on the α-carbon that impedes its β-oxidation and increases its half-life in lipid membranes. Interestingly, natural DHA hydroxyl derivates are also produced in the brain, such as neuroprotectin D1 (NPD1), and like DHA, NPD1 is also strongly diminished in the brain of AD patients [20]. The biological function of this molecule has been related to multiple neuroprotective effects, such as antioxidant, anti-inflammatory and anti-apoptotic roles. NPD1 also downregulates Aβ peptide production by modulating β- and α-secretase activities, and it favors neuronal survival against Aβ toxicity [20,21]. In the present work we found that OHDHA administration leads to enrichment of membranes in long PUFAs, which is associated with neuronal survival and neuroregeneration, so that OHDHA mimics the effects of NPD1. These changes in membrane lipid composition could facilitate the maintenance of a functional cell membrane structure that, in the case of AD, may reverse neurons from a pathological to a healthy condition [22]. Specifically, we show that by downregulating Aβ generation and Aβ-induced tau protein hyperphosphorylation, OHDHA promotes neuroprotection, and cell survival against different known AD-associated insults. In addition, this compound can also induce neuron stem cell proliferation via molecular and cellular mechanisms that remain largely unknown [19] and patients. Thus, OHDHA-induced neuron survival and proliferation should lead to improved cognition in AD models [19] and patients. In conclusion, OHDHA is presented here as a novel therapeutic candidate for the treatment of the AD-related neurodegeneration.

2. Materials and methods

2.1. Transgenic mice and treatments

A double transgenic PS1/APP mouse model was used in this work (5xFAD; line Tg6799) that harbors five human mutations associated to familial AD: the *Swedish* (K670N/M671L), *Florida* (I716V) and *London* (V717I) mutations in APP (amyloid precursor protein); and a human mutated PS1 (presenilin 1) harboring the M146L and L286V clinical mutations. Both these transgenes are expressed under control of the Thy-1 promoter. These mice display cognitive decline from 4 months of age [23]. These transgenic 5xFAD and wild type (WT) mice were purchased from Jackson Laboratories (USA), and they were maintained on a B6/SJL hybrid genetic background (C57BL/6 x SJL) by crossing heterozygous transgenic mice with B6/SJL WT (F1) breeders. All animals were housed at a controlled temperature and humidity (22 ± 2 °C; 70% humidity) on a 12 h–12 h light–dark cycle, and they were provided a standard laboratory diet ad libitum (Panlab A03; Barcelona, Spain).

WT and transgenic 5xFAD male mice were orally administered OHDHA (Lipopharma Therapeutics; Palma de Mallorca, Spain) dissolved in 5% ethanol at a dose of 15 mg/kg·day or the vehicle solution alone (5% ethanol; 15 ml/kg·day). These treatments started when the mice reached 3 months of age (dosed 5 days/week) and they were continued until the mice reached 7 months of age. During the last month of treatment, all the animals were submitted to a hypocaloric diet necessary to perform the selected behavioral spatial learning and memory test (food craving test in a radial arm maze) [24]. The results concerning the radial arm maze test have been reported previously [19], and a summary table (containing more relevant findings and total number of animals that were used for the test) has been also included in the discussion section of the present work (Table 4). Following the behavioral test, the mice were kept on normal diet (and treatment) for an additional week, after which they were euthanized, and their brain was removed immediately and dissected down the midline on a cold surface. Having removed the cerebellum, each cerebellum-free hemibrain was frozen in liquid nitrogen and stored at −80 °C. A total number of 9 animals were used in this work: 3 vehicle-treated WT, 3 vehicle-treated 5xFAD and 3 OHDHA-treated 5xFAD mice. All the protocols employed were approved by the Bioethical Committee of the University of the Balearic Islands, and they are in agreement with national and international regulations on animal welfare.

2.2. Lipid extraction and determination of cholesterol content

One hemi-brain from each animal was homogenized in a guanidine-salt buffer (RLT Buffer; Qiagen) at 1:20 (w:v) using a blade homogenizer (Polytron PT3100). The samples were then incubated at room temperature for 10 min and centrifuged for 5 min (10,000 *g*, 4 °C). The resulting supernatant was recovered, aliquoted and stored at −80 °C. Lipids were extracted using Bligh and Dyer's method [25]. The recovered organic phase was stored under a N_2 atmosphere at −80 °C. The cholesterol (Cho) content was determined as described previously [26]. Briefly, lipid extracts were evaporated under argon flow for at least 2 h and then resuspended in isopropanol. Total cho was measured in an aliquot (10 μl) using an enzymatic colorimetric kit (Biosystems; Barcelona, Spain) and determined through the appearance of a product absorbing at 500 nm.

2.3. Sphingolipid and phospholipid lipidomic analysis

Lipidomic studies were performed as described previously [27,28]. Liquid cromatography/mass spectrometry (LC/MS) analysis was performed on a Waters Aquity UPLC system connected to a Waters LCT Premier orthogonal accelerated time of flight mass spectrometer (Waters), operated in positive electrospray ionization mode. Full scan spectra from 50 to 1500 Da were acquired and individual spectra were summed to provide data points each 0.2 s. Mass accuracy and reproducibility were maintained by using an independent reference spray via LockSpray interference. The analytical column was a 100 × 2.1 mm inner diameter, 1.7 μm C8 Acquity UPLC bridged ethylene hybrid (Waters), thermostated at 30 °C. The two mobile phases both contained 5 mM ammonium formate: phase A, MeOH/H_2O/HCOOH (74:25:1, v/v); and phase B: MeOH/HCOOH (99:1, v/v). Quantification was carried out on 50 mDa windows using the extracted ion chromatogram of each compound, and the linear dynamic range was determined by injecting standard mixtures. Positive identification of compounds was based on accurate mass measurement, with an error of <5 ppm, and LC retention times compared to that of standards (±2%).