Omega-3 fatty acids and neuroinflammation in Alzheimer's disease: the unraveling of neurorestorative cell signaling

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Alzheimer's disease (AD) is a challenge to civilization due to its increasing incidence and the lack of preventive approaches and effective therapies. The challenges include huge emotional burdens to families and caregivers, as well as the growing economic liability to the health care system. Given the present increased life expectancy, age has become the number one risk factor for AD, and thus the incidence for this disease has grown because of it. The most prominent environmental risk factor for AD is traumatic brain injury and, in addition, several stroke and Parkinson's patients progressively transition to AD-type dementias. Currently there are no effective therapies for AD.

AD is a complex, multifactorial neurodegenerative disease characterized by progressive cognitive decline [1]. The early pathology includes enhanced neuroinflammation, which includes uncompensated oxidative stress and deregulated homeostasis with synaptic dysfunction and dendritic spine damage, followed by synaptic pruning and other events that lead to dementia. The

two main pathological characteristics of AD are (A) formation of intracellular neurofibrillary tangles of the hyperphosphorylated tau protein [2], and (B) accumulation of A β oligomers, leading to aggregates in amyloid plaques. These events are accompanied by microglia activation, astrogliosis and neurovascular unit impairments. Increased abundance of A β oligomers in the intercellular space are among the earliest defects in AD that mediate a chain of neurotoxic events initiated by synaptic dysfunction, post-synaptic dendritic spine loss, and finally overt neurodegeneration.

Docosahexaenoic acid (DHA), a member of the omega-3 essential fatty acid family, is concentrated uniquely in the CNS as a component of all membranes, particularly synaptic, dendritic and photoreceptors [3]. In contrast, arachidonic acid (AA, an omega-6 fatty acid) is distributed throughout mammalian tissues. DHA, which is esterified in phospholipids, follows a fate initiated by a phospholipase A2 (PLA2)-mediated cleavage. It then undergoes

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enzymatic oxygenation via several pathways, generating bioactive docosanoids that promote homeostasis and neurorestoration. Docosanoids includes: neuroprotection D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid), resolvins D1–D5 and maresins. 15-lipoxygenase-1 (15-LOX-1) is the enzyme that catalyzes the conversion of DHA into NPD1, a potent mediator of neuroprotection under conditions of uncompensated oxidative stress that, in turn, inhibits both proinflammatory and proapoptotic signaling enhancing prohomeostatic events (Figure 1).

Brain DHA content decreases during aging and particularly in AD. In the CA1 hippocampal areas from donors with early-stage AD (no major neuronal loss yet), DHA is diminished and NPD1 is selectively decreased along with 15-LOX-1, the enzyme that catalyzes NPD1 synthesis [4]. These assessments were made in brain tissue that carefully controlled plaque and tangle count, age- and sex-matched controls as well as postmortem sampling interval (within 3–4 h) [5]. A drop in NPD1 was also found in the hippocampus of 3xTg-AD mice between 4 and 12–13 months of age [5]. These observations were complemented by studies showing that the addition of NPD1 counteracted A β toxicity in human brain neurons in culture. The implication of these findings is that the inability of the AD brain to efficiently synthesize NPD1 perturbs the neuroprotective effectiveness of this defensive mechanism, which includes the ability of NPD1 to downregulate amyloidogenic APP cleavage to shift the processing of the APP to the neuroprotective sAPP- α , with concomitant decrease in the formation of toxic A β peptide [5]. These observations were made using human primary neuron/glia cultures overexpressing beta amyloid precursor protein (β APP)^{sw} (Swedish double mutation APP695^{sw}, K595N-M596L).

Thus NPD1 (and other docosanoids) are responsible for contributing at least in part to the bioactivity of DHA [6]. For example, NPD1 synthesis is upregulated as an early response to uncompensated oxidative stress, and to neurotrophins as specific adaptation-signaling responses, fostering resolution of inflammation and cell-survival signaling [3].

Because omega-3 fatty acids sustain brain function, prevent cognitive decline and are potentially beneficial in AD, the question to be asked is: why not supply them? This is followed by a more specific question: how can we supply

them? The epidemiology of omega-3 fatty acids in AD has been inconclusive due, in some part to AD disease heterogeneity. Since brain DHA decreases during aging, one way to counteract this deficit may be by supplying either 18:3, eicosapentaenoic acid or DHA. The potential benefits of DHA supplementation or increased dietary intake of fish in slowing down cognitive decline has yielded unclear results. For example, some clinical trials suggest that DHA plays a role in slowing cognitive decline in elderly individuals without dementia; however, there is no clear evidence of prevention or treatment of dementias, including AD. In one study showing lack of effects on AD, a subpopulation of patients with apoE4 polymorphisms did display benefits [7]. ApoE4 polymorphisms are increasingly recognized as a key factor in DHA significance in AD [8]. On the other hand, animal studies have shown that DHA ameliorates AD disease pathology [9,10]. Also, a dietary omega-3 fatty acid restriction in an AD mouse model resulted in loss of synaptic markers, whereas DHA administration reversed this neuronal deficit. Moreover, other studies have shown that a high-fat diet with low DHA content increases tau and A β levels, whereas a diet enriched in DHA reversed these changes, suggesting that shortage of DHA may be involved in the two main AD pathological features. Figure 1 depicts some of the beneficial bioactivity of NPD1: induction of a protective microglia phenotype as found in the retina [11]; induction of PPAR γ and sAPP α , as found in human brain cells [5]; transcriptional activation of nonredundant cRel/BIRC3 in the retina and brain [12]; activation of PP2A to down regulate oxidative stress-induce apoptosis at the mitochondrial level by dephosphorylation of Bcl-xL that, in turn, sequesters BAX [13]; and restoration of Tau mislocalization (speculative). In addition, astrocytes and microglia clearly are involved in AD [5,14].

Nonetheless, the complex regulation of lipid metabolism, and how this affects disease pathology, is far from understood. Disturbances in DHA, NPD1 or other lipid mediators are linked to the initiation of the disease, and administration of NPD1 could have an impact in lowering A β production and protection from neuronal insults. However, this might not be as straight forward as using dietary supplements of omega-3 fatty acids or DHA itself because between the ingestion and the uptake into the CNS are several events

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Figure 1. Counteracting pro- (blue) and anti- (red) homeostatic modulatory signaling in pathologies leading to cognitive decline and Alzheimer's disease. In the center is a membrane lipid mediator reservoir from where docosanoid precursors (e.g., DHA) are released either effectively or sluggishly. The docosanoid NPD1 is depicted, and pro- and anti-homeostatic targets are shown. One of the targets is microglia. One outcome is sustinment of the integrity of the synaptic circuitry. At the bottom are circuit impairments (depicts pruning of spines restored by NPD1) in an experimental model, the 5xFAD KO mouse (bottom right: VEH and NPD1 treatment in the 5xFAD KO mouse; protection of spines by the lipid mediator is shown). KO: Knockout; NPD1: Neuroprotectin D1; VEH: Vehicle.

not well understood that might be critical and would explain, at least in part, the apparently contradictory outcome of several clinical studies based on supplementation of omega-3 fatty acids. The brain and retinal DHA uptake involves not-well-understood steps followed from gastrointestinal absorption. For example, how omega-3 fatty acids interact with the gut microbiome is not known, but there is cross-talk between gut microbes and the immune

system that nurtures intestinal homeostasis. Impairments in the microbiome-host metabolic axis are involved in diseases, including neurodegenerations. Omega-3 fatty acids (18:3, 22:6, 22:5) are transported to the liver by the portal system and then transported to the brain and retina [15]. Therefore bioactive NPD1 shortage may not only be due to dietary restriction of the essential fatty acid precursor, but also to the liver as its supplier of building

blocks of excitable membranes that control the replenishment of the membrane stores. The liver enzymes that supply DHA to the brain are dysfunctional in AD, as reflected by the expression of peroxisomal d-bifunctional protein, which catalyzes the last step in the synthesis of DHA [16]. Moreover AD has multifactorial co-morbidities that include chronic systemic immune/inflammatory dysfunctions that heighten disease onset and progression [17,18].

There are some exciting evolving concepts regarding the overall line of thought developed in the editorial: (1) the molecular logic of neuroprotection and neurorestoration involves neuron- and astrocyte-evoked release of potent docosanoid mediators from cellular membrane reservoirs in response to specific challenges: uncompensated oxidative stress, onset of homeostasis disruptions, and of conditions that set in motion neurodegenerations [6]; (2) there is a membrane encoding of information susceptible to regulation by neurotrophins in terms of concerted responses that release specific docosanoid mediators in response to defined challenges; (3) there is a “molecular logic” in the decision-making process of retrieving the precursors (omega-3 fatty acids) of docosanoids and related mediators, which engages a genetically-programmed mechanism that defines the selective uptake/retention and storage specificity [19]; and (4) the failed adaptation response engages nonresolving inflammation set in motion by the perturbed balance of pro- and anti-homeostatic signaling quality and quantity of survival mediators (Figure 1). These fundamental issues of the biology of the CNS require a new scientific approach focused on neuronal cellular-adaptation failure as the inability to evoke sufficient, specific survival-inducing docosanoid mediators from cell membranes. Of course,

other mechanisms are critical, such as neurotrophin dysfunctions [20] and the incompletely-understood intracellular trafficking of proteins mediated by the retromer complex of proteins, among which mutations have been found in neurodegenerative diseases [21]. Therefore, the evolving concept is not that neural cell survival fails due to biology gone awry, but rather to specific mechanisms that may be therapeutically harnessed. Several apparently successful experimental drugs have failed advanced clinical trials, likely due to the complexity of the disease. We are currently realizing that clinical trial designs must include patient stratification as defined by genetic risks and disease phenotypes. An additional shortcoming has been the increasing recognition of the inadequacy of AD animal models for pre-clinical testing of therapeutics because, for example, the rodent genome is devoid of the APOE genes that are risk factors for sporadic AD.

In summary, omega-3 fatty acids, particularly DHA, clearly play a key role in neuroinflammation and Alzheimer's disease. The continued unraveling of the molecular logic of the cell signaling engaged will further our knowledge on novel, preventive and neurorestorative approaches for neurodegenerative diseases.

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