Lipid-Mediated Cell Signaling Protects against Injury and Neurodegeneration\textsuperscript{1,2}

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Abstract

Deficiency in docosahexaenoic acid (DHA) is associated with impaired visual and neurological development, cognitive decline, macular degeneration, and other neurodegenerative diseases. DHA is concentrated in phospholipids of the brain and retina, with photoreceptor cells having the highest DHA content of all cell membranes. The discovery that neuroprotectin D1 (NPD1; 10R, 17S-dihydroxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid) is a bioactive mediator of DHA sheds light on the biological importance of this fatty acid. In oxidative stress-challenged human retinal pigment epithelial (RPE) cells, human brain cells, or brain ischemia-reperfusion, NPD1 synthesis is enhanced as a response for sustaining homeostasis. Thus, neurotrophins, Abeta peptide (A\textsubscript{\textbeta}42, calcium ionophore A23187, interleukin-1beta (IL-1\beta)), or DHA supply enhances NPD1 synthesis. NPD1, in turn, upregulates the antiapoptotic proteins of the Bcl-2 family and decreases the expression of proapoptotic Bcl-2 family members. In human neural cells, DHA attenuates A\textsubscript{\textbeta}42 secretion, resulting in concomitant formation of NPD1. NPD1 repressed A\textsubscript{\textbeta}42-triggered activation of proinflammatory genes and upregulated the antiapoptotic genes encoding Bcl-2, Bcl-xl, and Bfl-1(A1) in human brain cells in culture. Overall, NPD1 signaling regulates brain and retinal cell survival via the induction of antiapoptotic and neuroprotective gene-expression programs that suppress A\textsubscript{\textbeta}42-induced neurotoxicity and other forms of cell injury. These in turn support homeostasis during brain and retinal aging, counteract inflammatory signaling, and downregulate events that support the initiation and progression of neurodegenerative disease.

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\textsuperscript{4} Abbreviations used: 15-LOX, 15-lipoxygenase; A\textsubscript{\textbeta}, Abeta peptide; AD, Alzheimer’s disease; \textalpha{\textbeta}PP, beta-amyloid precursor protein; cPLA\textsubscript{2}, cytosolic phospholipase A2\textsubscript{I}z; DHA, docosahexaenoic acid; HETE, hydroxyeicosatetraenoic acid; HN, human neuronal; IL-1\beta, interleukin-1beta; NPD1, neuroprotectin D1; PEDF, pigment epithelium-derived factor; RPE, retinal pigment epithelial; \textalpha{\textbeta}PP\textsubscript{x}, soluble amyloid precursor protein alpha; TNF, tumor necrosis factor.

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Introduction

There is growing awareness and interest in the biological importance of (n-3) fatty acids, particularly as they relate to brain development, vision, aging, and neurodegenerative diseases. The (n-3) fatty acid, docosahexaenoic acid (DHA),\textsuperscript{4} has its highest concentrations in the human body as an acyl group of phospholipids in the central nervous system, especially in photoreceptor discs and in synaptic membranes. DHA is necessary for retina and brain development (1,2), memory formation, synaptic function, and neuroprotection. This fatty acid has been implicated in several functions, such as those in photoreceptor biogenesis and function (3–5), memory (6), excitatory membranes functions (7), and neuroprotection (8). One property that the brain and retina share, with respect to (n-3) fatty acids, is their ability to retain DHA, even during extended dietary deprivation of (n-3) essential fatty acids. To efficiently reduce DHA content in brains and retinas of rodents and nonhuman primates, dietary deprivation for over 1 generation has been required, and the result was impaired retinal and brain function (9,10).

Epidemiological studies support the theory that (n-3) fatty acids slow down cognitive decline in the elderly (11). On the other hand, at least 11 observational studies and 4 clinical trials did not conclusively demonstrate that DHA plays a favorable role in the prevention or treatment of dementia, including Alzheimer’s disease (AD) (11). In AD transgenic mice, dietary DHA supplementation restored cerebral blood volume, reduced (Abeta peptide) A\textsubscript{\textbeta} deposition, ameliorated A\textsubscript{\textbeta} pathology (12–14), and downregulated A\textsubscript{\textbeta} release from aged human neural cells (15). DHA also exerts antiinflammatory and antiapoptotic actions (8,16,17).

Mechanisms underlying the protective actions of DHA are not well understood. DHA is prone to free-radical mediated peroxidation and enzyme-catalyzed oxygenation. Peroxidation products of DHA accumulate during brain ischemia-reperfusion...
and in neurodegeneration; these products in turn form protein adducts and other cytotoxic molecules that participate in free radical-mediated injury in AD (18,19). The identification of the DHA-derived docosanoid neuroprotectin D1 (NPD1:10R,17S-dihydroxy-docosa-4Z,7Z,11E,15E,19Z hexaenoic acid) provides new insight into the protective bioactivity of DHA (20–22). NPD1 elicits neuroprotective activity in brain ischemia-reperfusion (Fig. 1) in oxidative-stressed retinal pigment epithelial (RPE) cells, and it promotes neuronal and glial cell survival (21,22). DNA microarray profiling suggests a downregulation of pro-inflammatory genes as well as of some proapoptotic genes of the Bcl-2 family in cellular AD models (15). NPD1 further influences beta-amylloid precursor protein (βAPP) processing and the release of Aβ peptides, and its precursor DHA elicits an Aβ-lowering effect both in vitro and in vivo (23–25).

NPD1 is reduced in the AD cornu ammonis 1 hippocampal region and the neocortex, but not in other unaffected areas of the brain. The expression of key enzymes for NPD1 biosynthesis, cytosolic phospholipase A(2) [cPLA(2)], and 15-lipoxygenase (15-LOX) are altered in the AD hippocampal cornu ammonis 1 region (15).

**Neuroprotectin D1 inhibits ischemia-reperfusion-mediated leukocyte infiltration and stroke size**

Complexing DHA to human albumin after middle cerebral artery occlusion results in high-grade neurobehavioral and histological neuroprotection using a low albumin dose (0.63 g/kg), which in the absence of DHA is not robustly neuroprotective. The DHA–albumin complex also increases the production of NPD1 in the ipsilateral brain tissue (26,27).

**NPD1 downregulates interleukin-1β–activated amyloid β peptide secretion during in vitro aging of neural progenitor cells**

Human neuronal (HN) cells, a primary coculture of human neurons and glia, are a useful in vitro test system to explore stress signaling in the human brain, aging, and AD (28) (Fig. 2A). As indicated by βIII tubulin and glial fibrillary acidic protein immunostaining, these cultures are mixtures of equal proportions of neurons and glia under defined growth conditions at 4 wk of development (Fig. 2B, C). Interestingly, amyloidogenic Aβ peptides were progressively secreted from HN cells throughout 8 wk of culture (Fig. 2D, E). The ratio of Aβ40, a resident peptide of AD blood vessels, to Aβ42, which aggregates at lower concentrations than Aβ40 and is enriched within the amyloid plaque of AD (29), was ~10:1 throughout this 8-wk period. To determine the effect of cytokine-mediated stress on aging HN cells, Aβ40 and Aβ42 peptide release was assessed in the incubation medium after addition of interleukin (IL)-1β, an inducer of reactive oxygen species and of oxidative stress (28,30). A time-dependent release of both Aβ40 and Aβ42 peptides as a function of the number of weeks in culture was observed. Whereas soluble Aβ peptide secretion from HN cells was enhanced in the presence of interleukin-1beta (IL-1β), parallel experiments with DHA in the culture medium resulted in attenuation of Aβ peptide release (Fig. 2D, E).

Thus, HN cells use DHA as a precursor for NPD1 biosynthesis (Fig. 2F), yielding a 5-fold increase in NPD1 pool size at 4 wk of culture; at 8 wk, the concentration of this lipid mediator was about half that observed at 4 wk (Fig. 2F). These observations suggest that in aging HN cells, attenuation of the neurotoxic Aβ peptide release by DHA could be mediated, at least in part, by NPD1.

**sAPPα is an agonist for NPD1 synthesis**

Because DHA mediates the downregulation of Aβ40 and Aβ42 release and stimulates NPD1 production in HN cells, the possibility that NPD1 biosynthesis might be affected by the...
neurotrophic activity of sAPPα of added DHA. These results indicate that some of the stimulation strongly triggered NPD1 synthesis in the absence early in AD pathogenesis. sAPPα This may be a complementary cell survival mechanism activated by an upregulation in the biosynthesis of DHA-derived NPD1.

negligible NPD1 synthesis; however, at 100 values are means of 10, 20, 50, and 100 dependent. (H) NPD1 synthesis in HN cells, and this induction was age-dependent. (G) HN cells incubated in the presence of 10, 20, 50, and 100 g/L of sAPPα showed dose-dependent upregulation of NPD1 induction. In D-G, values are means ± SE, n = 6. In F, *different from corresponding −, P < 0.05. Adapted with permission from (15).

FIGURE 2 DHA attenuates Aβ peptide secretion and serves as the precursor for NPD1 biosynthesis. sAPPα activates NPD1 formation. (A) HN cells were grown for up to 8 wk. (B and C) After 4 wk of culture, aging HN cells displayed approximately equal populations of neurons and glia and stained positively with the (red fluorescent) neuron-specific marker βIII tubulin (B) and the (green fluorescent) glia-specific marker glial fibrillary acidic protein (C). (D and E) HN cells in culture normally release Aβ40 and Aβ42 peptides over 8 wk of aging. Secretion by HN cells of Aβ42 peptide was approximately one-tenth that of Aβ40 peptide; IL-1β (10 μg/mL in modified HN cell maintenance medium) increased, and DHA decreased, the release of both Aβ40 and Aβ42 peptides into the cell culture medium. CON, control. (F) DHA (100 nmol/L) induced NPD1 biosynthesis in HN cells, and this induction was age-dependent. (G) HN cells incubated in the presence of 10, 20, 50, and 100 g/L of sAPPα showed dose-dependent upregulation of NPD1 formation. In D-G, values are means ± SE, n = 6. In F, *different from corresponding −, P < 0.05. Adapted with permission from (15).

NPD1 counteracts Aβ peptide-induced apoptosis in neurons and glia

Because Aβ42 peptides induce apoptosis and cell death in both neurons and glia (35), the ability of NPD1 to protect HN cells against Aβ42-induced cytotoxicity was explored. For this purpose, 3-wk-old HN cells were incubated for an additional 3.5 d in serum-free HN cell maintenance medium containing 8 μmol/L in Aβ42 peptide. Except for the experiments depicted in Figure 2D–F, HN cells used in these studies were used at a developmental stage of 3–4 wk in culture, at which time there were approximately equal populations of neuronal and glial HN cells (Fig. 2A–C). Because selective cell loss may take place in older HN cell cultures (when neuronal cells drop out), the use of HN cells at a fixed age (and 50:50 neuronal/glial populations) was selected to
minimize this possibility. Apoptosis occurred in both neurons and glia. When NPD1 (50 nmol/L) was added to this test system, NPD1 protected both neurons and glia from Aβ-directed apoptosis, as evidenced by quantification of Hoechst 33258 staining of compacted nuclei in control, Aβ-treated, and Aβ+NPD1-treated cell fields. Unlike control HN cells, Aβ-treated HN cells also exhibited retracted neurites; however, when treated with NPD1, cells assumed extended neurites and an overall morphology resembling that of control cells.

15-LOX-1-catalyzed NPD1 synthesis mediates rescue from oxidative stress

During ischemia-reperfusion, aspirin enhances formation in the brain of 17R-resolvins, which are counterregulators of inflammation outside the brain. It could be argued that these DHA mediators enhance NPD1 bioactivity, decrease polymorphonuclear cell recruitment to the brain, and limit brain damage. Given the wide use of aspirin, the implied switch from endogenous to aspirin-triggered NPD1, which modulates homeostasis, is of great interest to neuraceuticals and dietary studies (21).

15-LOX-1-deficient RPE cells are more susceptible to perturbations that lead to apoptosis. The level of cell death is magnified in 15-LOX-1 deficient cells, as the concentration of oxidative stress mediators is increased. The magnitude of the response in the 15-LOX-1-deficient cells, in which the formation of NPD1 remained at low levels, was higher at 400, 600, and 800 μmol/L H2O2 [plus tumor necrosis factor (TNF)-α]. These observations, along with the unchanged content of 15-LOX-1, suggest that the diminished 15-LOX-1 activity, which forms lesser amounts of NPD1, contributes to increased apoptosis in knockdown cells. The increased sensitivity of RPE cells to oxidative stress-induced apoptosis may be due to a decreased availability either of NPD1 or of other 15-LOX-1 products. DHA and pigment epithelium-derived factor (PEDF) protect RPE cells synergistically from oxidative stress-induced apoptosis. NPD1 synthesis is enhanced under these conditions (22,36). To corroborate that NPD1 was in fact the 15-LOX-1 product responsible for protecting RPE knockdown cells, apoptosis induced in silenced cells using 600 μmol/L H2O2 and 10 μg/L TNFα was used to test protective bioactivity of PEDF and DHA, 15(S)-hydroxyeicosatetraenoic acid (HETE), 12(S)-HETE, lipoxin A4, or NPD1. Only NPD1 rescued 15-LOX-1-deficient cells from oxidative stress-induced apoptosis (Fig. 3). PEDF and DHA treatment was protective in nonsilenced cells but did not prevent apoptosis in the knockdown cells. Added DHA conversion to NPD1 is stimulated by PEDF; however, this conversion cannot take place in silenced cells. None of the arachidonic acid oxygenation products prevented cell death induced by oxidative stress (37).

Concluding remarks

The interplay of DHA-derived neuroprotective signaling aims to counteract proinflammatory, cell-damaging events triggered by...
multiple, converging cytokine and amyloid peptide factors in AD. Amyloid peptide-mediated oxidative stress, the activation of microglia associated with Aβ peptide deposition, and excessive production of microglial-derived cytokines, such as IL-1β and TNFα, support progressive inflammatory episodes in AD (35,38–40). These noxious stimuli further orchestrate pathogenic gene-expression programs in stressed brain cells, thereby linking a cascade of caspase-mediated cell death pathways with apoptosis and neuronal demise (28,41). Neural mechanisms leading toward NPD1 generation from DHA thereby appear to redirect cellular fate toward successful brain cell aging (Fig. 4). The Becl-2 pro- and antiapoptotic gene families, sAPs, and NPD1 lie along a cell fate-regulatory pathway whose component members are highly interactive and have potential to function cooperatively in brain cell survival during aging and during the onset of neurodegeneration. Overall, they operate in large part through modulation of Aβ42-directed pathogenic events. Taken together, these data suggest that NPD1 induces an antiapoptotic, neuroprotective gene-expression program that regulates the secretion of Aβ peptides, resulting in the modulation of inflammatory signaling, neuronal survival, and the preservation of brain cell function. Agonists of NPD1 biosynthesis or NPD1 analogs may be useful for exploring therapeutic strategies for AD and related neurodegenerative disease.

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C.Z. and N.G.B designed the research, wrote the paper, and are responsible for the final content.

Literature Cited


