Docosahexaenoic Acid and Its Derivative Neuroprotectin D1 Display Neuroprotective Properties in the Retina, Brain and Central Nervous System

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Abstract
The significance of the selective enrichment in omega-3 essential fatty acids (docosahexaenoyl – DHA – chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (e.g. synaptic membranes and dendrites) has remained, until recently, incompletely understood. While studying mechanisms of neuronal survival, we contributed to the discovery of a docosanoid synthesized by 15-lipoxygenase-1 from DHA, which we dubbed neuroprotectin D1 (NPD1;10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). NPD1 is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20C arachidonic acid family of essential fatty acids not enriched in the nervous system. We found that NPD1 is promptly made in response to oxidative stress, seizures and brain ischemia-reperfusion. NPD1 is neuroprotective in experimental brain damage, retinal pigment epithelial cells, and in human brain cells. Thus, NPD1 acts as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations. NPD1 also has been shown to have a specificity and potency that provides beneficial bioactivity during initiation and early progression of neuronal and retinal degenerations, epilepsy and stroke. In short, NPD1 regulation promotes homeostatic regulation of neural circuitry integrity.

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Introduction

The interface between the immune and inflammatory systems actively influences the development and maintenance of the central nervous system (CNS) by sustaining homeostasis. This interface is critical to preserve the cellular and functional integrity of the nervous system during aging as well. Moreover, most diseases and dysfunctions of the nervous system display failures in early responses from the immune and inflammatory systems.

The selective uptake and avid retention of the omega-3 essential fatty acid (FA; fig. 1) docosahexaenoic acid (DHA) by the CNS is a salient characteristic necessary for synaptogenesis as well as development and biogenesis of all neuronal cellular membranes. This also includes photoreceptors since the retina is an integral part of the CNS. Unlike members of the omega-6 FAs (e.g. arachidonic acid), which are present in all parts of the body, DHA is selectively taken up by the CNS and is needed for a variety of functions, including memory and vision. DHA is concentrated and retained in the nervous system and becomes part of the membranes. DHA is also used in development of cellular elements that comprise the neurovascular unit, the innate immune resident cells, the microglia, and the astrocytes.

During the last few years, bioactive messengers derived from DHA have been identified, and these discoveries have opened up a renewed interest in understanding the bioactivity and significance of this essential FA in the nervous system.

The first bio-derivative of DHA, neuroprotectin D1 (NPD1; 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid) was found to be made on-demand in the CNS. NPD1 biosynthesis is activated at the onset of various injuries, including ischemia-reperfusion damage, protein misfolding, Aβ peptide challenge, enhanced oxidative stress, and others (fig. 1). This stereospecific mediator is secreted upon synthesis, extending its neuroprotective properties to the vicinity of the injury. These characteristics, along with the prohomeostatic form of action that NPD1 elicits, make it a useful and interesting molecule for the development of new therapies to treat neurodegenerative diseases.

Docosahexaenoic Acid and Docosanoids

Docosanoids are derived from 22C DHA and include NPD1, maresins [1], neuroprostanes, and related 22C derivatives, unlike eicosanoids, which are derived from the 20C arachidonic acid family of essential FAs (fig. 1). Previous studies
Fig. 1. Omega-3 and omega-6 FA mechanisms of cell death and survival. Phospholipids carrying both omega-3 and omega-6 FAs form part of the cellular membrane. DHA with a chain of 22 carbons is avidly retained in cell membranes of the CNS as opposed to arachidonic acid (AA; 20 carbons) that occurs ubiquitously. Phospholipase A₂ (PLA₂) hydrolyzes both FA types from the second position in the phospholipids of the plasma membrane, and they are converted into bioactive signaling lipids. AA is the precursor of prostaglandins, prostacyclins, thromboxanes, and leukotrienes, among others. Cyclooxygenase-1 (COX-1) and COX-2 are the main enzymes that convert AA into their bioactive lipids. These immunologically related messengers form part of the signaling, which mediates cellular response and inflammation, acting through specific receptors to elicit their bioactivity. Peroxidation products of AA and DHA produce 4-hydroxynonenal (4-HNE) and 4-hydroxyhexenal (4-HHE), which are toxic byproducts of generalized oxidative stress. On the other hand, 15-LOX-1 is the enzyme that converts DHA into its stereospecific dioxygenation product NPD1. NPD1 has stereospecific binding that triggers modulation of proinflammatory and proapoptotic processes by altering the expression and phosphorylation state of Bcl-2 proteins COX-2, CEX-1 and B-94. It also enhances the activation of pro-survival key kinases such as Akt. Altogether, the activity of NPD1 promotes corneal nerve regeneration as well as survival of neuronal and other retinal cells. The synthesis of NPD1 is greatly enhanced by the action of neurotrophins, including PEDF and BDNF.
identified the formation of mono-, di-, and trihydroxy DHA derivatives in the retina. These studies show that lipoxygenase inhibitors block synthesis of these derivatives, indicating an enzymatic process of a lipoxygenase nature [2]. Although the stereochemistry and bioactivity of DHA-oxygenated derivatives were not determined at that time, it was proposed that these DHA derivatives could be neuroprotective, and thus the term ‘docosanoids’ was coined [3, 4]. Liquid chromatography-photodiode array-electrospray ionization tandem mass spectrometry-based lipidomic analysis was then used to identify oxygenation pathways for synthesis of NPD1 during brain ischemia-reperfusion [5], in epilepsy, in the retinal pigment epithelium (RPE) [6], in the human brain [7], and in other cells [8, 9].

**Docosahexaenoic Acid and Neuroprotectin D1**

The stereospecific isomer NPD1 is derived from the enzymatic dioxygenation of DHA (fig. 1). Photoreceptors, RPE cells, and the brain contain unesterified (free) DHA under basal, nonstimulated conditions [10–14], suggesting that the pool size of unesterified DHA is tightly regulated by phospholipase A2, reacylation, and peroxidation under some conditions. Free DHA becomes the substrate of docosahexaenoyl-coenzyme A synthesis for channeling through acyltransferases, which incorporate this FA into phospholipids [15–18]. RPE cells therefore modulate the uptake, conservation, and delivery of DHA to the photoreceptors [4]. In addition, RPE cells use a specific pool of DHA phospholipids to initiate NPD1 synthesis by 15-lipoxygenase-1 (15-LOX-1) [19] (fig. 1).

NPD1 induces homeostatic/pro-survival signaling on cells undergoing stress as a result of cellular and systemic damage [3, 5, 7]. For instance, NPD1 induces neuroprotection in brain ischemia-reperfusion and in oxidative-stressed retinal cells [5, 20, 21] and inhibits retinal ganglion cell death [21], is protective in kidney ischemia-reperfusion and regulates adiponectin [22]. In addition, NPD1 upregulates antiapoptotic proteins (Bcl-2 and Bcl-xL) and downregulates proapoptotic proteins (Bax and Bad) in response to cellular oxidative stress and cytokine activation leading to an overall pro-survival transcriptome [3, 5–7].

NPD1 also provides the basis to understand the mechanisms by which DHA promotes survival during neuroinflammation and neurodegeneration as it is the mediator that executes protective bioactivity of DHA in the CNS. Deficiency of NPD1 and 15-LOX-1, the enzyme involved in its formation, has been observed in Alzheimer’s disease brain [7]. Also, NPD1 further influences β-amyloid precursor protein processing and decreases amyloid-β (Aβ 42 release [23]. DHA,
the precursor of NPD1, elicits an Aβ$_{42}$-lowering effect both in vitro and in vivo [24–26].

Conversely, DHA effects differ from NPD1-triggered ones in some instances. For example, it is worthy to note that free radical-mediated DHA peroxidation products accumulate during ischemia and neurodegeneration (fig. 1). These oxidation products in turn may form protein adducts and other cytotoxic molecules that promote further free radical injury [27–29]. Enzymatic dioxygenation of DHA, as opposed to peroxidation, produces stereospecific products, such as NPD1, that are controlled, produced and secreted [19]. NPD1 also stereo-selectively modulates pathways triggered by insults such as oxidative stress in femtomolar doses [19]. Thus, NPD1 activity may explain at least in part the pro-survival properties of DHA.

**Docosahexaenoic Acid and Neuroprotectin D1 in Photoreceptor Cell Membranes**

Photoreceptor cell damage and apoptotic death are tell-tale signs of retinal degenerative diseases [30–32]. In retinitis pigmentosa (RP), rod photoreceptor death begins on the edges of the retina, while in age-related macular degeneration (AMD) progressive agitation and vision loss result from photoreceptor death in the macula, the center of the retina [33]. Retinal degenerations include several cell-signaling pathways, such as various triggers of photoreceptor cell death. This death is closely linked with the relationship between photoreceptors and RPE cells. For example, photoreceptors are damaged in diseases such as Stargardt’s disease and other retinal degenerations where RPE cell integrity is compromised. If RPE cells die, photoreceptor cells also succumb [34]. There are more than 150 mutations of photoreceptor-specific proteins reported to contribute to RP, including mutations of rhodopsin, peripherin, the β-subunit of cGMP phosphodiesterase, and photoreceptor outer segment membrane protein 1 (ROM1) [35, 36]. In autosomal-dominant RP, some of the genes involved are expressed in the choriocapillaris [37]. AMD, however, is a group of complex disorders with multiple environmental and genetic causes. The main known risk factor is advanced age. Moreover, the choriocapillaris and Bruch’s membrane, which separates the RPE from the choriocapillaris, also are affected in AMD [38]. Initiation and progression of this retinal degeneration involves an unsuccessful inflammatory response. Recent findings have identified major risk factors for AMD: single nucleotide polymorphisms in the genes encoding factor H (CFH/HF1) [39], factor B (BF), and complement component 2 (C2) [40]. Factor H is an inhibitor of the alternative pathway of complement system activation
that limits cell injury and inflammation [41]. Nevertheless, our knowledge on the mechanisms leading to these diseases is limited.

Complex intracellular and intercellular signaling events are set in motion during the preliminary stages of retinal degeneration. During these stages, oxidative and nitrosative stress is increased and exaggerated, usually at abnormal cellular locations and with inappropriate timing. Mitochondrial function is also compromised, and this dysfunction is a major factor in these impediments [37, 42, 43].

NPD1 has also been shown to inhibit choroidal neovascularization (CNV) in a laser-induced mouse model of CNV [44]. This model shows how NPD1 protects photoreceptor cells from apoptosis while downregulating the proinflammatory cytokines TNF-α and IL-1β, possibly by inhibiting NF-κB activity and reducing cyclooxygenase-2, leading to decreased VEGF expression. Overall, NPD1 may contribute to induction of the resolution phase of the inflammatory response (fig. 1). The antiangiogenic and neuroprotective actions of NPD1 make it an excellent candidate for treating wet AMD and diabetic retinopathy.

Because early clinical expressions of most retinal degenerations precede photoreceptor cell death, it is important to determine these early critical events. This knowledge may lead to new therapeutic interventions for halting or slowing disease progression.

**Docosahexaenoic Acid and Neuroprotectin D1 in Stroke**

Stroke is the third leading cause of death and the first cause of adult disability in the United States. In spite of the knowledge gained regarding stroke pathophysiology, the only treatment approved for ischemic stroke is thrombolysis, and only 3–5% of patients can undergo this treatment. Thus, there is a critical need for developing effective stroke treatments.

Cerebral ischemia results in rapid accumulation of free FAs, such as DHA released from membrane phospholipids. DHA is concentrated in phospholipids of various cells of the CNS [11] and is processed in the liver for transport to various tissues by the bloodstream. DHA release from cell membranes mostly occurs under conditions of excessive oxidative stress [11]. As an acyl chain of membrane phospholipids, DHA is necessary for ion channels, receptors, and transporters to maintain proper physical properties [11]. It is involved in memory formation [45], excitable membrane function [46], and neuroprotection [47, 48].

As mentioned previously, DHA is the precursor of NPD1, which downregulates apoptosis and promotes cell survival [6, 49, 50]. In addition, some studies
show that NPD1 inhibits brain ischemia-reperfusion-mediated leukocyte infiltration and proinflammatory gene expression [49]. It also promotes in vitro and in vivo neurogenesis [5] (fig. 1). NPD1 displays potent anti-inflammatory actions and protects against brain ischemia-reperfusion [5] and oxidative stress [3, 6, 7].

Recent compelling evidence gathered in our lab shows the neuroprotective properties of DHA when it is systemically administered in a rat model of middle cerebral artery occlusion (MCAo). DHA improves neurological function in rats and reduces the extent of histological damage with a therapeutic window of at least 3 h after MCAo onset. As such, DHA offers great promise in developing therapies for cerebral ischemia in patients with acute ischemic stroke. Again, one of the possible mechanisms of neuroprotection mediated by DHA may involve its conversion into bioactive lipid mediators such as NPD1.

Docosahexaenoic Acid and Neuroprotectin D1 in Epilepsy

Temporal lobe epilepsy is a disorder where hippocampal function is disrupted by neuronal hyperexcitability [51–53], often spreading from the hippocampus to other areas of the brain [54].

Under resting conditions, unesterified DHA is present in the brain in small amounts. However, when seizures induce the activation of phospholipases, esterified DHA is released and it accumulates in hippocampal synaptosomes [55]. DHA has been proposed to elicit anti-seizure activity through modulation of ion channels and neurotransmitter receptors, as well as regulation of synaptic plasticity [56–59].

Progressive and permanent enhancement of local and propagating seizure activity occurs in response to repeated hippocampal stimulation in a kindling epileptogenesis model for temporal lobe epilepsy [60–63]. Here, DHA and NPD1 modulate the hippocampal electrical activity associated with evoked seizures, thereby limiting the progression of focal limbic seizures. Also, DHA significantly increases seizure latency induced by pentylenetetrazol (PTZ) [64]. The evidence suggests that NPD1 may attenuate the onset of PTZ-induced seizures and ameliorate enhanced electrical activity. In this promising field, future studies are required to determine the effect of oral and systemic DHA administration on seizure frequency in different experimental epilepsy models for potential translational research. As such, both DHA and NPD1 make attractive targets in the development of therapeutic approaches for treating and preventing epilepsy.
Conclusions

Omega-3 FAs are essential for the body, and supplementation may have important implications for human health, particularly in the CNS. Dietary supplementation may positively influence dyslipidemia, atherosclerosis, hypertension, diabetes mellitus, obesity, metabolic syndrome, and other inflammatory diseases. Fish, fish oils, and some vegetable oils are rich sources of omega-3 FAs. On the other hand, a recent prospective analysis does not support the concept that long-term dietary intake of omega-3 FAs decreases the risk of type 2 diabetes. In contrast, higher fish and omega-3 FA consumption appears to be associated with higher incidence of type 2 diabetes. Given the beneficial effects of fish and omega-3 FAs on multiple risk factors associated with diabetes, including triglycerides, high-density lipoprotein cholesterol, blood pressure, and inflammation, and on coronary heart disease, the major sequela of diabetes, the clinical relevance of this relation and its possible mechanisms require further investigation.

Ultimately, clinical trials using various DHA formulations will need to demonstrate the safety and efficacy of these interventions. Because omega-3 FAs (mainly DHA) are liable to peroxidation, DHA signalolipidomics research on the molecular details of DHA uptake, disposition, and action will likely identify other factors/molecules/systems that protect DHA integrity, particularly during the initiation and early progression of diseases. These new approaches may lead to novel multinutrient-based trials in the future.

Both DHA and its stereospecific derivative NPD1 have been shown to have neuroprotective roles in the retina and CNS. Future studies are needed to help garner information for developing therapies to conquer diseases such as RP, AMD, stroke, epilepsy, Alzheimer’s and other neural and retinal degenerative diseases.

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Disclosure Statement

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References


60 Young C, Gean PW, Chiou LC, Shen YZ: Docosahexaenoic acid inhibits synaptic transmission and epileptiform activity in the rat hippocampus. Synapse 2000;37:90–94.


