Vision and neurological function supported by the potent, stereospecific mediator neuroprotectin D1 biosynthesized from docosahexaenoic acid

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Abstract

The neuroprotective properties of omega-3 fatty acids have been demonstrated during the years by several laboratories. The identification of neuroprotectin D1 (NPD1), a biosynthetic product of docosahexaenoic acid (DHA), in brain and retina as well as the characterization of its bioactivity, is generating a renewed interest on the omega-3 fatty acid's functional role and physiopathological significance.

In experimental stroke, endogenous NPD1 synthesis was found to be upregulated, and the infusion of the lipid mediator into the brain under these conditions revealed neuroprotective bioactivity of NPD1. Furthermore, when DHA was administered i.v. after middle-cerebral artery occlusion, protection was concomitant with NPD1 synthesis on the ipsilateral brain side. In the retinal pigment epithelial (RPE) cells, NPD1 synthesis induction results in cytoprotection against oxidative stress. The bispyridinium bisretinoid, A2E (a byproduct of phototransduction that becomes toxic when it accumulates in RPE cells during aging or in age-related macular degeneration), when added to RPE cells, was found to display attenuated cytotoxicity in the presence of NPD1. Integrity of RPE cells is necessary for photoreceptor cell survival and vision.

Neurotrophins, particularly pigment epithelium-derived factor (PEDF), induce NPD1 synthesis and its polarized apical secretion, implying paracrine and autocrine bioactivity of this lipid mediator. Moreover, DHA elicits a concentration-dependent and selective potentiation of PEDF-stimulated NPD1 synthesis and release through the apical RPE cell surface. The signaling activated by PEDF and DHA uncovered synergistic cytoprotection, with concomitant NPD1 synthesis, when cells were challenged with oxidative stress. Also, DHA and PEDF synergistically activate anti-apoptotic protein expression and decreased pro-apoptotic Bcl-2 protein expression and caspase 3 activation during oxidative stress. Thus, our results identify neurotrophins as regulators of NPD1 biosynthesis and of its polarized apical efflux from RPE cells. Moreover, phagocytosis of photoreceptor outer segments by retinal pigment epithelial cells downregulates oxidative stress-mediated apoptosis with concomitant synthesis of NPD1.

The homeostatic regulation between photoreceptors and RPE preserves RPE cell integrity during successful aging. In fact, RPE cell density is maintained during nine decades if eye pathology does not arise. However, failure of homeostasis results in enhanced DHA peroxidation, drusen formation, lipid peroxide protein adduct accumulation, apoptosis, and pathoangiogenesis. Overall, it is apparent that a breakdown in the balance of protective and potentially cytotoxic factors is involved in various forms of retinal degeneration. NPD1

synthesis is induced under conditions where excessive oxidative stress threatens to disrupt homeostasis, and rescue signals, such as neurotrophins, are released to protect cell integrity.

Hippocampal CA1 regions from Alzheimer's disease (AD) patients (relatively rapidly sampled) show a major reduction in NPD1. Based upon this finding, we developed a human brain cell aging model to study further the significance of NPD1. The aging of human neural progenitor cells (HN cells, neurons and glia) in primary culture during 8 weeks is accompanied by an 8-fold enhanced synthesis and release of Aß40 and Aß42 peptides that resembles Aß deposition during brain aging and in AD. IL-1ß stimulates gamma-secretase-mediated cleavage of ßAPP into Aß peptides. Conversely, DHA suppressed both Aß40 and Aß42 peptide release with concomitant NPD1 synthesis. Moreover, NPD1 inhibits Aß42-induced apoptosis in HN cells. Therefore, DHA neuroprotection in aging human brain cells involves NPD1 synthesis.

Pro- and antiapoptotic proteins are modulators proximal to mitochondria and cell damage. Proapoptotic Bik and Bax were enhanced by Aß42, but not by DHA or NPD1, whereas Bcl-2, Bcl-xl, and Bfl-1(A1) were increased in the presence of DHA. NPD1, on the other hand, promoted a much larger increase in antiapoptotic Bcl-2 proteins. Bfl-1(A1) increased almost 6-fold. These modulatory actions of NPD1 may play critical roles in the survival of aged and terminally differentiated cells and break the mechanistic link between inflammatory signaling and apoptosis. In fact, NPD1 also induces the antiapoptotic Bcl-2 family proteins Bcl-2 and Bcl-xl in oxidatively challenged human retinal pigment epithelial cells and promotes cytoprotection. Thus 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.

Neural mechanisms leading toward NPD1 generation from DHA thereby appear to redirect cellular fate toward successful preservation of RPE-photoreceptor cell integrity, and brain cell aging. The Bcl-2 pro- and antiapoptotic gene families, sAPP alpha (and/or other neurotrophins) and NPD1, lie along a cell fate–regulatory pathway whose component members are highly interactive, and have potential to function cooperatively in brain and retina cell survival. Agonists of NPD1 biosynthesis, NPD1 analogs or dietary regimens may be useful for exploring new preventive/therapeutic strategies for neurodegenerative diseases.

BAZAN, N. G. 2007. Vision and neurological function supported by the potent, stereospecific mediator neuroprotectin D1 biosynthesized from docosahexaenoic acid. *Biol. Skr. Dan. Vid. Selsk.* **56**: 75-81. ISSN 0366-3612 • ISBN 978-87-7304-327-1

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Key words: vision, neuroprotection, neuroprotectin D1, docosahexanoic acid

Introduction

The neuroprotective properties of omega-3 fatty acids have been demonstrated during the years by several laboratories. The identification of neuroprotectin D1 (NPD1), a biosynthetic product of docosahexaenoic acid (DHA), in brain and retina as well as the characterization of its bioactivity (Bazan, 2005; 2006), is generating a renewed interest on the omega-3 fatty acid's functional role and physiopathological significance. In experimental stroke, endogenous NPD1 synthesis was found to be upregulated (Marcheselli *et al.,* 2003), and the infusion of the lipid mediator into the brain under these conditions revealed neuroprotective bioactivity of NPD1 (Marcheselli *et al.*, 2003). Furthermore, when DHA was administered i.v. after middle-cerebral artery occlusion, protection was concomitant with NPD1 synthesis on the ipsilateral brain side (Belayev *et al.,* 2005).

Retinal pigment epithelial (RPE) cells, human progenitor cells, and brain models of injury (e.g., ischemia-reperfusion) respond to oxidative stress or injury by activating the synthesis of an endogenous neuroprotective mediator, NPD1 (Fig. 1) (Mukherjee *et al.,* 2004). The name 'neuroprotectin D1' was suggested based upon its **neuroprotect**ive bioactivity in oxidatively stressed RPE cells and brain, and its potent ability to **in**activate pro-apoptotic and pro-inflammatory signaling. 'D1' refers to its being the first identified neuroprotective mediator derived from DHA. DHA belongs to the omega-3 essential fatty acid family (all of which are derived from linolenic acid, 18:3, ω-3) and, therefore, cannot be made *de novo* in the body. The photoreceptor cells, unlike most other cells of the nervous system, are highly enriched in DHA, tenaciously retaining DHA even during very prolonged periods of omega-3 fatty acid deprivation (Bazan, 2006; SanGiovanni and Chew, 2005; Marszalek and Lodish, 2005).

Previous studies have shown that the retina forms mono-, di-, and trihydroxy derivatives of DHA, and lipoxygenase inhibitors block this synthesis, suggesting an enzymatic process of a lipoxygenase nature (Bazan *et al.,* 1984). Although, at the time, the stereochemistry and bioactivity of these DHA-oxygenated derivatives were not defined, it was proposed that these lipoxygenase products might be neuroprotective (and at the same time, the name 'docosanoids' was suggested) (Bazan *et al.,* 1984; 1985). Upon the advent of liquid chromatography, photodiode array, electrospray ionization, and tandem mass spectrometry-based lipidomic analysis, a collaboration between the group of Charles Serhan (Harvard Medical School) and our group identified oxygenation pathways for the synthesis of the docosanoid NPD1 during brain ischemia-reperfusion (Marcheselli *et al.,* 2003). Moreover, it was also found that RPE cells have the ability to synthesize NPD1 (Mukherjee *et al.,* 2004). NPD1 is formed from free (unesterified) DHA and released from membrane phospholipids by a phospholipase A2 (PLA2). Photoreceptors and RPE cells, although they contain phospholipids richly endowed with DHA (as docosahexaenoyl- or DHA-elongated fatty acyl-chains), display an undetectable quantity of unesterified (free) DHA (as is the case with unesterified arachidonic acid) under basal, unstimulated conditions (Bazan, 2003; Aveldano and Bazan, 1974; 1975; Horrocks and Farooqui, 1994; Sun *et al.*, 2004). This means that the pool size of unesterified DHA is tightly controlled at the levels of its production by a PLA2, by its removal (e.g., by reacylation), and by peroxidation. Free DHA to be incorporated into membrane phospholipids first becomes the substrate of docosahexaenoyl-coenzyme A synthesis for its channeling through acyltransferases that incorporate this fatty acid into phospholipids (Reddy and Bazan, 1984a; 1984b; 1985a; 1985b). The RPE cell thus modulates the uptake, conservation, and delivery of DHA to photoreceptors (Bazan *et al.,* 1985). In addition, the RPE cell utilizes a specific DHA-phospholipid pool as a precursor

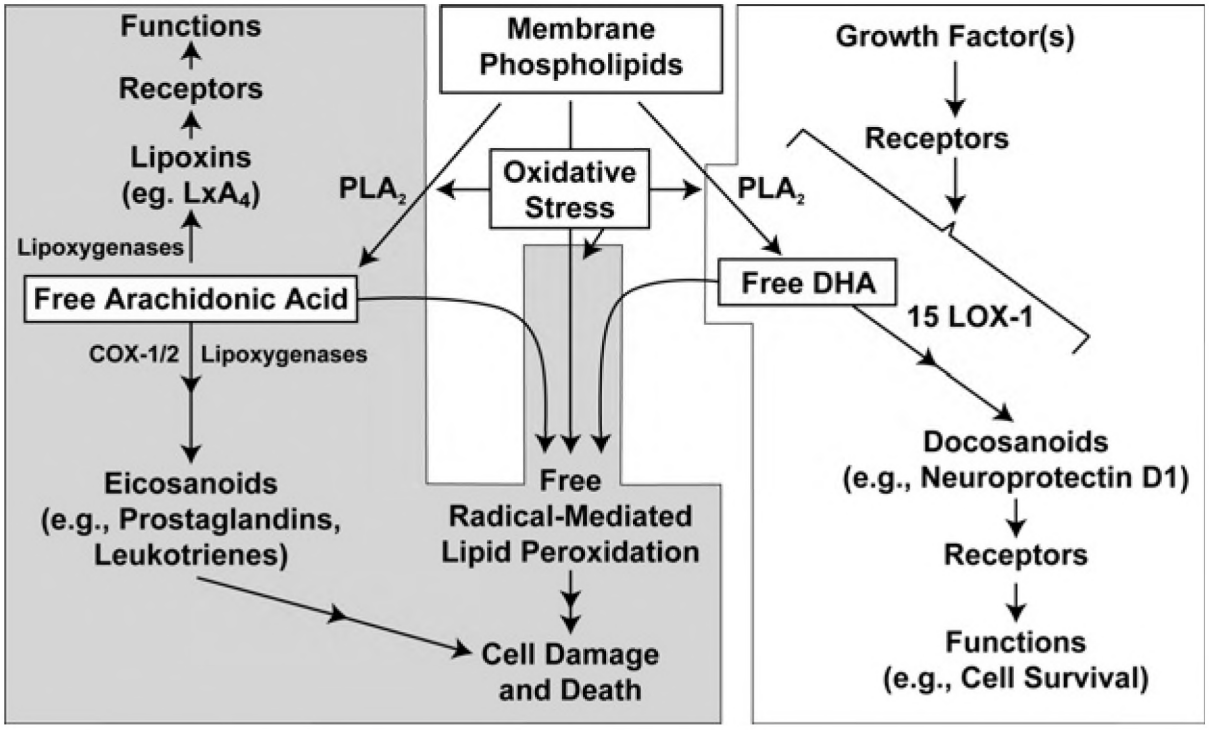

Fig. 1. Fate and bioactivity of derivatives of poly-unsaturated acyl chains (arachidonic acid, C20:4, ω-6; docosahexaenoic acid, C22:6, ω-3) of membrane phospholipids. PLA2 = phospholipase A2. The central arrows indicate that free radical-mediated lipid peroxidation may attack esterifed arachidonoyl or docosahexaenoyl chains, as well as free arachidonic or docosahexaenoic acids. The evolving lipid peroxidation products are highly reactive and promote cell injury. On the left of the diagram (shaded box), phospholipase A2 is depicted releasing free arachidonic acid and leading to the eicosanoid cascade. In the top left portion of the blue box, lipoxin synthesis is indicated. This pathway down regulates inflammation. In the lower portion of the blue box, other eicosanoids may contribute to enhancing inflammation. On the right side of the diagram (unshaded box), this figure illustrates that growth factor-mediated activation of the synthesis of docosanoids leads to biologically active mediators such as neuroprotectin D1, which in turn operate through receptors. Oxidative stress by itself activates both docosanoid synthesis (to counteract cytotoxic actions) and free radical-mediated lipid peroxidation.

for the pathway leading to NPD1 synthesis. Then this stereospecific mediator is synthesized after DHA is released through DHA oxygenation by a PLA2, followed by a 15-lipoxygenase-like activity (Mukherjee *et al.,* 2004). The nature of these enzymes needs to be precisely defined. In Alzheimer's disease (AD) brain of short postmortem time, it was found that cPLA2α and 15 lipoxygenase-1 expression changed in concert with NPD1-decreased content and DHA enhanced pool size in the CA1 area of the hippocampus (Lukiw *et al.,* 2005). In ARPE-19 cells (spontaneously transformed human RPE cells), interleukin (IL)-1β, oxidative stress, or the Ca^{2+} ionophore A23187 activates the synthesis of NPD1 (Mukherjee *et al.,* 2004). In turn, NPD1 might act in an autocrine fashion and/or diffuse through the IPM, to act in a paracrine mode on photoreceptor cells and/or Müller cells (Bazan, 2006).

Neuroprotectin D1: a homeostatic regulator of photoreceptor/retinal pigment epithelial cell integrity

In the RPE cells, NPD1 synthesis induction results in cytoprotection against oxidative stress (Mukherjee *et al.*, 2004). The bispyridinium bisretinoid, A2E (a byproduct of phototransduction that becomes toxic when it accumulates in RPE cells during ageing or in age-related macular degeneration), when added to RPE cells, was found to display attenuated cytotoxicity in the presence of NPD1. Integrity of RPE cells is necessary for photoreceptor cell survival and vision (Bazan, 2006). Neurotrophins, particularly pigment epithelium-derived factor (PEDF), induce NPD1 synthesis and its polarized apical secretion, implying paracrine and autocrine bioactivity of this lipid mediator. Moreover, DHA elicits a concentration-dependent and selective potentiation of PEDF-stimulated NPD1 synthesis and release through the apical RPE cell surface. The signaling activated by PEDF and DHA uncovered synergistic cytoprotection, with concomitant NPD1 synthesis, when cells were challenged with oxidative stress. Also, DHA and PEDF synergistically activate anti-apoptotic protein expression and decreased pro-apoptotic Bcl-2 protein expression and caspase 3 activation during oxidative stress. Thus, our results identify neurotrophins as regulators of NPD1 biosynthesis and of its polarized apical efflux from RPE cells (Mukherjee *et al.*, 2007a). Moreover, phagocytosis of photoreceptor outer segments by retinal pigment epithelial cells downregulates oxidative stress-mediated apoptosis with concomitant synthesis of NPD1 (Mukherjee *et al.*, 2007b).

The homeostatic regulation between photoreceptors and RPE preserves RPE cell integrity during successful ageing. In fact, RPE cell density is maintained during nine decades if eye pathology does not arise (Gao and Hollyfield, 1992). However, failure of homeostasis results in enhanced DHA peroxidation, drusen formation, lipid peroxide protein adduct accumulation, apoptosis, and pathoangiogenesis. Overall, it is apparent that a breakdown in the balance of protective and potentially cytotoxic factors is involved in various forms of retinal degeneration (Bazan, 2006; Rattner and Nathans, 2006; Bok, 2005; Strauss, 2005). NPD1 synthesis is induced under conditions where excessive oxidative stress threatens to disrupt homeostasis, and rescue signals, such as neurotrophins, are released to protect cell integrity (Fig. 1). Triggers of the NPD1 response include A2E and A2E epoxides (oxiranes) that accumulate in the ageing RPE and in Stargardt's disease and other retinal degenerations (Sparrow and Cai, 2001). Therefore, NPD1 is a potent modulator of photoreceptor/RPE cell functional integrity (Bazan, 2007).

Neuroprotectin D1 promotes cell survival during neurodegeneration

Hippocampal CA1 regions from AD patients (relatively rapidly sampled) show a major reduction in NPD1 (Belayev *et al.*, 2005). Based upon this finding, we developed a human brain cell ageing model to study further the significance of NPD1. The ageing of human neural progenitor cells (HN cells, neurons and glia) in primary culture during 8 weeks is accompanied by an 8-fold enhanced synthesis and release of Aß40 and Aß42 peptides that resembles Aß deposition during brain ageing and in AD. IL-1ß stimulates gamma-secretase-mediated cleavage of ßAPP into Aß peptides. Conversely, DHA suppressed both Aß40 and Aß42 peptide release with concomitant NPD1 synthesis. Moreover, NPD1 inhibits Aß42-induced apoptosis in HN cells. Therefore, DHA neuroprotection in ageing human brain cells involves NPD1 synthesis.

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Conclusions

The molecular and functional organization of the cell is being further explored by genomics, proteomics, and metabolomics. Within metabolomics, lipidomics is an evolving and powerful approach for the detailed identification of lipid classes and molecular species, including structural lipids as well as bioactive lipids (mediators of cell signaling). The lipidome of a cell, or part of a cell (e.g., dendrites, specific cellular compartment), defines the complete characterization of the lipids. We are using a lipidomic-based analysis for the decoding of omega-3 fatty acids in the central nervous system. This approach has led to the discovery of neuroprotectin D1 in the RPE cell (Mukherjee *et al.,* 2004) and the uncovering of its bioactivity.

Overall the interplay of DHA-derived neuroprotective signaling (NPD1-mediated) aims to counteract proinflammatory, cell-damaging events triggered by multiple, converging cytokine and amyloid peptide factors in AD (Lukiw, 2005). Neural mechanisms leading toward NPD1 generation from DHA thereby appear to redirect cellular fate toward successful preservation of RPE-photoreceptor cell integrity (Bazan, 2006; 2007), and brain cell ageing (Lukiw, 2005). The Bcl-2 pro- and antiapoptotic gene families, sAPP alpha (and/or other neurotrophins) and NPD1, lie along a cell fate–regulatory pathway whose component members are highly interactive, and have potential to function cooperatively in brain and retina cell survival. Agonists of NPD1 biosynthesis, NPD1 analogs or dietary regimens may be useful for exploring new preventive/therapeutic strategies for neurotrauma, stroke, and neurodegenerative diseases.

Acknowledgements

Supported by National Institutes of Health, National Eye Institute grant EY005121, National Institute of Neurological Disorders and Stroke grants NS023002 and NS046741, and National Center for Research Resources grant P20 RR016816.

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