Neuroprotectin D1 (NPD1): A DHA-Derived Mediator that Protects Brain and Retina Against Cell Injury-Induced Oxidative Stress

Nicolas G. Bazan

LSU Neuroscience Center and Department of Opthamology, Louisiana State University Health Sciences Center School of Medicine in New Orleans.

Corresponding author:
Nicolas G. Bazan, LSU Neuroscience Center, 2020 Gravier Street, Suite D, New Orleans, LA 70112, USA (E-mail: nbazan@lsuhsc.edu)

The biosynthesis of oxygenated arachidonic acid messengers triggered by cerebral ischemia-reperfusion is preceded by an early and rapid phospholipase A_2 activation reflected in free arachidonic and docosahexaenoic acid (DHA) accumulation. These fatty acids are released from membrane phospholipids. Both fatty acids are derived from dietary essential fatty acids; however, only DHA, the omega-3 polyunsaturated fatty acyl chain, is concentrated in phospholipids of various cells of brain and retina. Synaptic membranes and photoreceptors share the highest content of DHA of all cell membranes. DHA is involved in memory formation, excitable membrane function, photoreceptor cell biogenesis and function, and neuronal signaling, and has been implicated in neuroprotection. In addition, this fatty acid is required for retinal pigment epithelium cell (RPE) functional integrity. Here we provide an overview of the recent elucidation of a specific mediator generated from DHA that contributes at least in part to its biological significance. In oxidative stress-challenged human RPE cells and rat brain undergoing ischemia-reperfusion, 10,17*S*-docosatriene (neuroprotectin D1, NPD1) synthesis evolves. In addition, calcium ionophore A23187, IL-1β, or the supply of DHA enhances NPD1 synthesis. A time-dependent release of endogenous free DHA followed by NPD1 formation occurs, suggesting that a phospholipase A_2 releases the mediator's precursor. When NPD1 is infused during ischemia-reperfusion or added to RPE cells during oxidative stress, apoptotic DNA damage is down-regulated. NPD1 also up-regulates the anti-apoptotic Bcl-2 proteins Bcl-2 and BclxL and decreases pro-apoptotic Bax and Bad expression. Moreover, NPD1 inhibits oxidative stress-induced caspase-3 activation. NPD1 also inhibits IL-1β-stimulated expression of COX-2. Overall, NPD1 protects cells from oxidative stress-induced apoptosis. Because photoreceptors are progressively impaired after RPE cell damage in retinal degenerative diseases, understanding of how these signals contribute to retinal cell survival may lead to the development of new therapeutic strategies. Moreover, NPD1 bioactivity demonstrates that DHA is not only a target of lipid peroxidation, but rather is the precursor to a neuroprotective signaling response to ischemia-reperfusion, thus opening newer avenues of therapeutic exploration in stroke, neurotrauma, spinal cord injury, and neurodegenerative diseases, such as Alzheimer disease, aiming to up-regulate this novel cell-survival signaling.

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INTRODUCTION

Dietary omega-3 fatty acids are required to maintain cellular functional integrity, and overall they are necessary to human health (60). Docosahexaenoic acid (22:6, n-3, DHA), a major component of fish oil and marine algae, is most highly concentrated in photoreceptors and brain and retinal synapses (8). Other neural cells, such as glia, are also richly endowed with this fatty acid. DHA is esterified mainly in C2 of the glycerol backbone of phospholipids. Although DHA is present in all cells, it attains the highest concentrations in brain and retina. On the other hand, arachidonic acid is the prevalent polyunsaturated fatty acyl chain of most cell phospholipids.

Arachidonic acid in phospholipids is the reservoir of biologically active eicosanoids. Only very recently has it been shown that DHA in phospholipids is also a reservoir for biologically active mediators, the docosanoids. Both polyunsaturated fatty acids are a target for free radical-mediated peroxidation as well (Figure 1). Free AA and DHA are released from membrane phospholipids through the action of phospholipases in response to stimulation (eg, neurotransmitters, brief, non-cell-damaging seizures), ischemia, neurotrauma, etc. (9, 27, 64). This response tells us that phospholipases are an important regulatory gate-keeper in the initiation of the eicosanoid and docosanoid pathways under both physiologic and pathologic conditions. During basal cell function, there is an active, ATP-dependent reacylation of free AA and DHA in membrane phospholipids (Figure 2). Oxidative stress in brain generates neuroprostanes from DHA, through an enzyme-independent reaction (50). DHA clearly is required for brain and retina development (5), and has been implicated in several functions, including those of excitable membranes (32, 54), memory (19, 38, 55), photoreceptor biogenesis and function (1, 2, 17, 44, 63, 66), and neuroprotection (29). One property of retina and brain (insofar as their omega-3 fatty acids are concerned) is their unusual DHA-retention ability, even during prolonged dietary deprivation of essential fatty acids of the omega-3 family. To effectively reduce the content of DHA in retina and brain in rodents and in non-human primates, dietary deprivation for more than one generation has been required (42, 65), which produces impairments of retinal function (41, 66).

The studies on DHA-mediated neuroprotection in photoreceptors (47, 52) and brain (28) have not identified a mediator or a mechanism. Thus the following questions have emerged. Is the pro-survival action of DHA the result of replenishing the fatty acid into membrane phospholipids? Or is it due to a more selective signaling by a DHA-derived mediator? This article is an overview on the elucidation of specific DHA mediators that elicit neuroprotection.

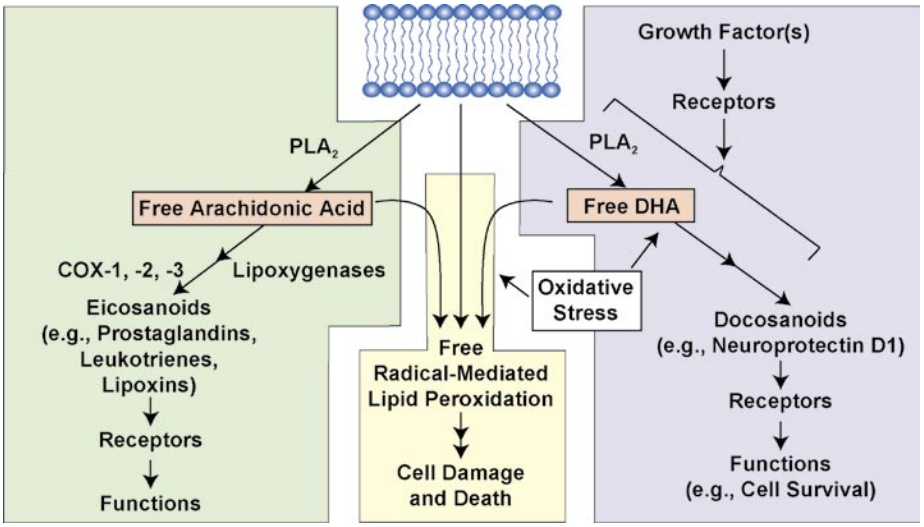

Figure 1. Fate of polyunsaturated acyl chains of membrane phospholipids. PLA_2 = phospholipase A_2. The central arrows (in yellow box) indicate that free radical-mediated lipid peroxidation may attack esterifed arachidonoyl or docosahexaenoyl chains, as well as free arachidonic or docosahexaenoic acids (left and right arrows). The evolving lipid peroxidation products are highly reactive and promote further cell injury. On the left of the diagram (green), phospholipase A_2 is depicted releasing free arachidonic acid and leading to the eicosanoid cascade that modulates cell functions through specific receptors. On the right side of the diagram (blue), it is suggested 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.

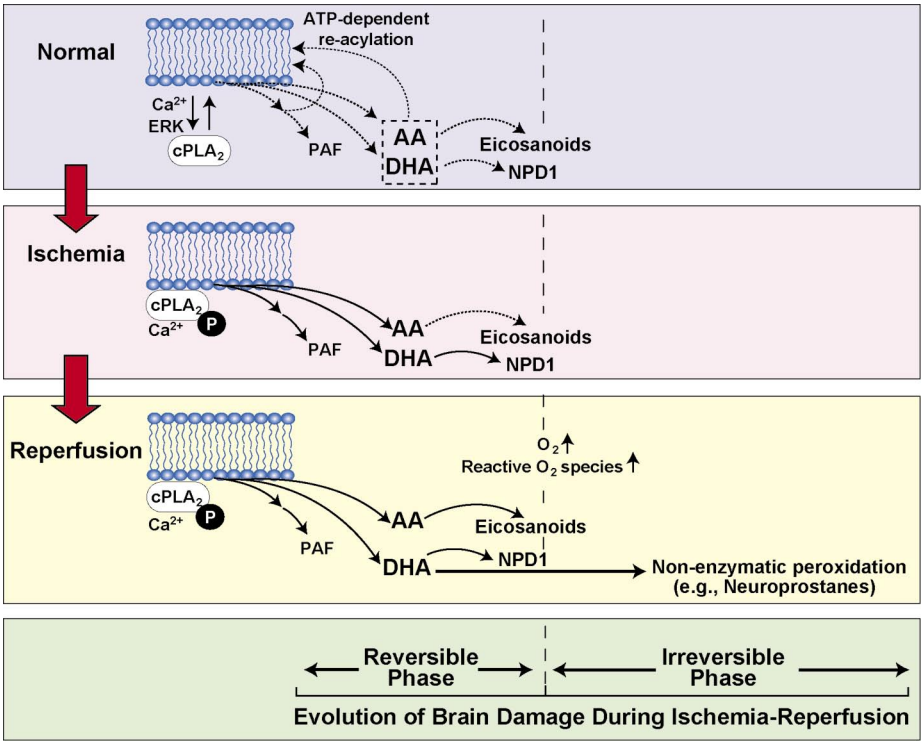

Figure 2. Brain ischemia-reperfusion-induced activation of free arachidonic (AA) and docosahexaenoic (DHA) acid release as well as of platelet-activating factor (PAF). Under physiologic conditions, the pool sizes of these free fatty acids is negligible because they are highly regulated. During ischemia, PLA_2 is activated and free AA and DHA as well as PAF are accumulated. While oxygen lasts, NPD1 may be formed to cope with early ischemic consequences. During reperfusion the surge in O_2 promotes the formation of reactive O_2 species and non-enzymatic lipid peroxidation. Early in this phase, NPD1 synthesis is activated (34). The extent of synthesis of neuroprotective mediators such as NPD1 may delineate the reversible phase of brain ischemia-reperfusion damage. Thereafter, the predominance of irreversible cell-damaging events comprises the irreversible phase.

BRAIN AND RETINA CONTAIN THE HIGHEST CONTENT OF DHA OF ANY TISSUE

Diet-supplied DHA or its precursor 18:3, n-3 are initially taken up by the liver and then distributed through blood lipoproteins to meet the needs of organs, notably during synaptogenesis and photoreceptor cell biogenesis (12, 56). The precursor 18:3, n-3, is elongated and desaturated to DHA in the liver, thus most of the liver efflux of omega-3 fatty acids is DHA itself (56). Modulation of the uptake of DHA from the microcirculation is effected by the neurovascular unit in brain and by the choriocapillaris and retinal pigment epithelial (RPE) cells in retina. The latter route is the major one that supplies DHA and other nutrients to photoreceptor cells. The neural retina proper has a network of capillaries that nourishes mainly the non-photoreceptor cells. The details of the cellular and molecular events in tissue DHA uptake remain to be fully elucidated. Once DHA arrives to the nervous tissue, astrocytes seem to regulate its uptake and distribution, although this process is also not well understood. The fate of DHA at the cell level is to become activated in the form of DHA-CoA by acyl CoA synthetases, followed by ATP-dependent acyltransferases that introduce the fatty acid mainly into the C2 position of the glycerol backbone of phospholipids.

Photoreceptor outer segments contain rhodopsin as well as the highest content of DHA of any cell type (2, 8, 21). The photoreceptor tips are in intimate contact with a monolayer of cells derived from the neuroepithelium, the retinal pigment epithelial cells, which are the most active phagocytes of the human body. In a daily cycle these cells phagocytize the distal tips of photoreceptor outer segments, promoting rod outer segment renewal (28) in a tightly controlled process of addition of new membrane to the base of the outer segments. DHA, esterified in the phospholipids of photoreceptor membranes, is retained through rod outer segment renewal. The conservation of DHA in photoreceptors is mediated by retrieval through the interphotoreceptor matrix, which supplies the fatty acid for the biogenesis of outer segments (14, 24, 63). The daily renewal of photoreceptors is regulated so that their length and chemical composition, including that of their phospholipids, are maintained. Photoreceptor

phospholipids contain most of their DHA in carbon 2 of the glycerol backbone, but they also display molecular species of phospholipids with DHA in both C1 and C2 positions of the glycerol backbone (5, 6, 7, 21, 67).

NPD1 IS AN ENZYME-DERIVED NEUROPROTECTIVE MEDIATOR FROM DHA

NPD1 is formed through enzyme-mediated steps catalyzed by a phospholipase A_2 followed by a 15-lipoxygenase-like activity (Figure 1). It has been known that retina is able to generate mono-, di-, and trihydroxy derivatives of DHA, and that lipoxygenase inhibitors block this synthesis, suggesting an enzymatic process involving a lipoxygenase (13). Although the precise stereochemistry of these DHA-oxygenated derivatives was then unknown, it was suggested that docosanoids might be endowed with neuroprotective bioactivity (8). In brain and retina, the availability of free (unesterified) DHA is tightly controlled. In fact, the DHA pool size in these tissues is negligible under basal, unstimulated conditions (3). Thus, the regulation of the phospholipase A_2 that releases free DHA plays an important role in the pathway leading to the formation of NPD1. The significance of this step is highlighted by the very rapid activation of free DHA release in brain as an initial response to stimulation, ischemia or seizures (9, 27, 64).

Using brain undergoing ischemia-reperfusion (34), ARPE-19 cells (40), and primary cultures of human RPE cells (unpublished observations) in combination with tandem LC-PDA-ESI-MS-MS-based lipidomic analysis, we isolated and structurally characterized 10,17S-docosatriene (NPD1). ARPE-19 cells are spontaneously transformed human retinal pigment epithelial cells that conserve cell biological and functional properties (46). The newly isolated dihydroxy-containing DHA derivative from RPE and brain cells is called "neuroprotectin D1" (NPD1): *i)* because of its neuroprotective properties in brain ischemia-reperfusion (34) and in oxidative stress-challenged RPE cells (40), *ii)* because of its potent ability to inactivate pro-apoptotic signaling (40), and *iii)* because it is the first identified neuroprotective mediator of DHA. NPD1 is a stereospecific mediator derived through a docosahexaenoic acid-oxygenation pathway.

The calcium ionophore A23187, or to a lesser extent, IL-1β, activates the synthesis of NPD1 in a time-dependent manner, with a concomitant increase in the endogenous free DHA pool size that is 3- to 4-fold higher than the amount of NPD1 being synthesized (40). The RPE cell actively recycles DHA from phagocytized disc membranes, where DHA is concentrated in membrane phospholipids, back to the inner segment of the photoreceptor cell (14). The RPE cell's ability to synthesize NPD1 is a newly identified function of this cell (40). NPD1 may act in autocrine fashion and/or may diffuse through interphotoreceptor matrix proteins and act in paracrine fashion on photoreceptor cells or in other cells. NPD1 bioactivity may be elicited through a receptor, and in turn down-regulate the induction of NF-κB and of other transcription factors, and as a consequence, decrease pro-inflammatory gene expression (34). In addition, NPD1 intracellular signaling modulates Bcl-2 family protein availability (40).

Oxidative stress triggers multiple signaling pathways, including some that are cytoprotective and others that contribute to cell damage and eventually cell death. Among these are the Bcl-2 family proteins. In fact, expression of pro- and anti-apoptotic Bcl-2 family proteins is altered by oxidative stress, and these proteins are a major factor in the outcome of apoptotic signaling, since cell survival reflects the predominance of one set of proteins over the other (36). In the RPE, retina, and brain, oxidative stress promoted by several factors including various forms of injury or reactive oxygen species shifts the balance of the Bcl-2 family protein expression toward those that favor cell damage (31, 36, 45). These modifications in the expression of Bcl-2 proteins imply that the early response to oxidative stress includes transcriptional, translational, and/or post-translational events upstream of the mitochondrial apoptotic step. In this connection oxidative stress-triggered ARPE-19 cell damage includes changes in the availability of Bcl-2, Bcl-xL, Bax, and Bad. It is conceivable that oxidative stress, cytokines, and other intercellular signals including growth factors may activate NPD1 formation, in an effort to counteract the injury/pro-inflammatory response and restore homeostasis. Exogenous NPD1 promotes a differential modification in the expression of Bcl-2 family proteins under these conditions, up-regulating the protective Bcl-2 proteins and attenuating the expression of the proteins that challenge cell survival, particularly Bax and Bad (40). These observations suggest a critical coordinate regulation of the availability of Bcl-2 proteins for subsequent downstream signaling. NPD1 may act at the level of signaling that regulates promoters of the genes encoding death repressors and effectors of the Bcl-2 family of proteins. The elucidation of the precise molecular mechanisms will provide an important insight into regulatory survival signaling. Bcl-2 family proteins regulate apoptotic signaling at the level of the mitochondria and endoplasmic reticulum. As a consequence, cytochrome c is released from mitochondria and effector caspase-3 is activated. In agreement with this sequence, oxidative stress-activated caspase-3 in ARPE-19 cells is decreased by NPD1. Interestingly, NPD1 was also very effective in counteracting oxidative stress-induced apoptosis in ARPE-19 cells. This action was not mimicked by eicosanoids such as PGE_2, LTB_4, or arachidonic acid. This supports the selectivity of the new class of mediator, NPD1. Perhaps one of the most interesting observations is that DHA itself inhibited oxidative stress-induced apoptosis. Under those conditions, a remarkable, time-dependent formation of NPD1 occurred (40). Significantly, the potency of DHA for cytoprotection was much higher than that of added NPD1, suggesting that endogenously generated NPD1 may exert its action near the site of its synthesis. Alternatively, it may imply that other NPD-like mediators may be formed and participate in promoting RPE cell survival. It is indeed possible that related NPD mediators are generated in an attempt to cope with the multiplicity of cellular signaling that has the potential of going awry in RPE or neurons when confronted with oxidative stress. In support of this possibility, brain does make a series of other potentially bioactive DHA-oxygenated derivatives such as those generated in the presence of aspirin during ischemia-reperfusion (34).

The functions of RPE include the transport and reisomerization of bleached visual pigments, the maintenance of the integrity of the blood-outer retinal barrier, and

the handling of DHA uptake from the blood stream and its continuous resupply (or recycling) to photoreceptors. The RPE cell takes up DHA from the blood stream through the choriocapillaris, and in turn supplies the fatty acid to photoreceptors through the interphotoreceptor matrix (14). This uptake is very active during early postnatal development, when photoreceptor outer segment biogenesis occurs (56). In addition, active docosahexaenoyl CoA synthases in the RPE and the retina channel free DHA to acyltransferases that incorporate the fatty acid into membrane phospholipids (49). The RPE cell thus is very active in the uptake, conservation, and delivery of DHA to photoreceptors (8, 14). Retinal detachment or trauma sets into motion dysfunctions in the RPE cells that lead to the onset and development of proliferative vitreoretinopathy.

RPE cells are critical for photoreceptor survival, and oxidative stress-mediated injury and cell death in RPE in turn impair vision, particularly when the RPE cells of the macula, which is responsible for visual acuity, are impaired. The pathophysiology of many retinal degenerations (eg, age-related macular degenerations, Stargardt disease) involves oxidative stress leading to apoptosis of RPE cells (48, 59, 62).

DHA-CONTAINING PHOSPHOLIPIDS ARE A TARGET OF FREE RADICAL-CATALYZED PEROXIDATION

Brain and retinal DHA is a target of oxidative stress-mediated lipid peroxidation (4). In the case of brain ischemia-reperfusion, lipid peroxidation participates in neural injury (10, 15). DHA, esterified in membrane phospholipids, is released in brain ischemia (9, 27, 64, 70), and is thought to yield lipid peroxides (50) (Figure 1). Leukocyte infiltration and pro-inflammatory gene expression are mediators of ischemic stroke damage (35, 51, 53). Leukocytes release myeloperoxidase and this in turn promotes further production of free radical reactive molecules. The biosynthesis of oxygenated arachidonic acid messengers (23, 39) triggered by cerebral ischemia-reperfusion is preceded by an early and rapid phospholipase A_2 activation reflected in free arachidonic and docosahexaenoic acid accumulation (4, 9, 27, 64, 70). The excessive activation of this event promotes further neural damage. Thus free radical-catalyzed lipid peroxidation is a major, deleterious set of cell reactions.

ISCHEMIA-REPERFUSION BRAIN DAMAGE

Brain undergoing ischemia-reperfusion generates stereospecific DHA-oxygenation pathways that lead to the formation of novel messengers: the first pathway is responsible for the synthesis of 10,17*S*-docosatriene (NPD1), and the second pathway, which is active in the presence of aspirin, leads to the formation of the resolvin-type messengers (17*R*-DHA). These pathways have the potential for exerting counter-regulatory actions on cellular and molecular signaling that promotes brain injury. In the presence of aspirin and during ischemia-reperfusion, characteristic DHA messengers are formed that in non-neural tissues are potent mediators of inflammation resolution (58). These resolvin-type DHA-derived messengers may elicit additional neuroprotective actions in brain ischemia-reperfusion. NPD1 is a potent inhibitor of ischemia-reperfusion-induced PMN infiltration and pro-inflammatory gene induction. This novel DHA messenger also inhibits cytokine-mediated pro-inflammatory gene activation in neural cells in culture. Overall, NPD1 potently elicited neuroprotection in vivo by reducing the stroke infarct volume after middle cerebral artery occlusion (MCAO).

It is important to note that, in the presence of aspirin, there was enhanced formation in brain of 17*R*-series resolvins that have recently been found to be cytoprotective and counter-regulators of inflammation outside the nervous system (57). It could be argued that these compounds enhance the actions of endogenously generated docosanoids, which are the counter-regulatory substances generated from DHA to decrease leukocyte recruitment to brain and to limit leukocyte-mediated inflammation and brain damage. The implied switch, from endogenous to aspirin-triggered, DHA-derived lipid mediators that enhance this protective action, is of great interest for future studies, given the wide use of aspirin.

The synthesis of NPD1 after one hour of MCAO coincides with free DHA availability that results from phospholipase A_2 activation (9, 10, 27, 64, 70), as well as with reperfusion reoxygenation. The endogenous brain synthesis of NPD1 that peaks at 8 hours of reperfusion may be a response of insufficient magnitude to counteract leukocyte infiltration and pro-inflammatory gene induction under these conditions. Thus the relatively large ischemic insult produced by one hour of MCAO followed by hours of reperfusion may overcome the ability of the endogenously generated docosanoids to elicit neuroprotection (34). Thus exogenous administration of NPD1 directly into the cerebroventricular system through continuous infusion during the initial 2 days of reperfusion did indeed exert neuroprotection. From 24 to 72 hours of reperfusion is when most brain leukocyte infiltration occurs (20, 22, 35, 53). Leukocytes accumulate in an area surrounding the brain infarct, and are thought to possess a multifactorial ability to promote injury in brain ischemia-reperfusion (25). Moreover, PMN infiltration as well as amoeboid microglia along the edges of the stroke infarct may be responsible for expansion of the penumbra region, which occurs from 16 hours onwards after MCAO/reperfusion (33). NPD1 continuously administered by intracerebroventricular perfusion inhibited such infiltration, and subsequently reduced the stroke volume by 50% after 48 hours. The amount of docosanoid infused was one μg over a 48-hour period, at a rate of 250 nl/h. This observation implies that NPD1 is neuroprotective. Once leukocytes infiltrate the brain, they release IL-1β, TNFα, and other cytokines, as well as myeloperoxidase, which in turn catalyzes the formation of additional reactive oxygen species. Myeloperoxidase in brain intercellular spaces is potentially a highly effective enzymatic catalyst for the initiation of lipid peroxidation (71), and brain cells are richly endowed with a major target of that process, polyunsaturated fatty acyl phospholipids (8, 10).

As in RPE, during oxidative stress, brain ischemia releases unesterified DHA (4, 9, 27, 64, 70), and during early stages of ischemia reperfusion, endogenous NPD1 is synthesized. The bioactivity of NPD1 at the cellular level is characterized by potent anti-inflammatory actions. We have studied IL-1β because it increases during brain ischemia-reperfusion as a result of PMN infiltration as well as activation of microglia and macrophages (33). Whether the bioactivity of NPD1 in inhibiting PMN infiltration and blocking pro-inflammatory gene expression are independent events or

part of the same signalling remains to be defined. What is clear is that the outcome of infusing NPD1 is neuroprotection from ischemia-reperfusion damage.

NEURODEGENERATIVE DISEASES: ALZHEIMER AND AGE-RELATED MACULAR DEGENERATIONS

Decline of memory and cognition in Alzheimer disease (AD) is underlain by untimely synaptic pruning, dendritic degeneration, enhanced inflammatory signaling, neurofibrillary tangles, senile plaques, and neuronal loss. The accumulation of extracellular amyloid beta (Aβ) protein from membrane bound beta amyloid precursor protein (βAPP) in the limbic system is characteristic of the senile plaques of AD, although there is still debate as to whether or not Aβ accumulation and its consequences fully explains the AD phenotype. Aβ peptides promote pro-inflammatory responses and are activators of neurotoxic pathways (11, 68, 69). These events include enhanced excitotoxicity via increased calcium flux into neurons, activation of brain microglia, overproduction of reactive oxygen species, and an overall oxidative stress response in the brain (11, 37, 69). In contrast, a soluble APP-alpha (sAPPα) peptide, generated from βAPP via the α-secretase pathway, decreases the production of neurotoxic Aβ peptides and elicits neurotrophic and synaptotrophic effects.

DHA is preferentially concentrated within membrane phospholipids of the central nervous system (CNS), especially at central synapses and in retinal photoreceptors (34, 40, 69). Free DHA is liberated by a stringently regulated phospholipase A_2 (PLA_2), and may subsequently be converted into 10,17S- docosatriene (NPD1) through an enzyme-mediated lipoxygenation via a 15-lipoxygenase (15-LOX)-like enzyme. We characterized NPD1 in mouse brain ischemia-reperfusion and identified and characterized the neuroprotective properties of this lipid mediator (34). There is an interrelationship between brain membrane-derived Aβ42, sAPPα, DHA and NPD1 signaling and we have examined their effects on the aging and fate of stressed human neural cells in primary culture. The endogenous bioactive DHA-derived lipid NPD1 may be a key regulator of an intrinsic neuroprotective and anti-apoptotic gene expression program that promotes brain cell survival.

The high content of DHA in photoreceptors and RPE has been linked to date mainly to endowing photoreceptor membrane domains with physical properties that contribute to the modulation of receptors (eg, rhodopsin), ion channels, transporters, etc. For example, in other cells DHA modulates G-protein-coupled receptors and ion channels. Moreover, DHA has been suggested to regulate membrane function by maintaining its concentration in phosphatidylserine (55). DHA is also envisioned as a target of oxidative stress, mainly by reactive oxygen intermediates that generate DHA-peroxidation products and in turn participate in RPE and photoreceptor cell damage.

Rhodopsin mutations in retinitis pigmentosa expressed in rats are associated with a decreased content of DHA in photoreceptors (2). This observation is interpreted as a possible retinal response to a metabolic stress, whereby decreasing the amount of the major target of lipid peroxidation, DHA, contributes to protection (2). Alternately, the retinal DHA pool size available for synthesis of neuroprotective docosanoids may be compromised due to lipid peroxidation. Retinal degeneration induced by constant light promotes DHA loss from photoreceptors, and rats reared in bright cyclic light are protected (30). These studies suggest adaptation/plasticity that may involve endogenous molecules (30) that have not been characterized to date. Some of these may be lipid mediators, such as NPD1.

Are growth factors involved in the survival-promoting activity of NPD1? In this connection, FGF2 induces bovine RPE cell survival in cultures through a sustained adaptive phenomenon that involves ERK2 activation by secreted FGF1 and ERK2-dependent Bcl-xL production (18). Bcl-xL may play a key role in integrating and transmitting exogenous FGF2 signals for RPE cell survival. Issues such as this remain to be explored.

A consequence of RPE cell damage and apoptosis is impaired photoreceptor cell survival, a dominant factor in age-related macular degeneration (26). For example, in Stargardt disease (a juvenile form of macular degeneration), oxidative stress mediated by the lipofuscin fluorophore A2E produces RPE damage and caspase-3 is part of the damaging cascade, whereas Bcl-2 exerts cellular protection (61).

THERAPEUTIC IMPLICATIONS OF OMEGA-3 FATTY ACIDS AND NPD1

The potent bioactivity of NPD1 in promoting neuronal and RPE cell survival, when these cells are confronted with injury, is the basis for exploring the receptors involved, as well as for identifying by structure/activity relationships the active part of this lipid mediator. To date, we have also taken another approach: we have explored the delivery of DHA during ischemia-reperfusion through the systemic circulation. In these studies, we have taken advantage of the high-affinity binding sites for DHA of human serum albumin. In addition, our studies were encouraged by the finding that human serum albumin is indeed neuroprotective under these conditions (16). Our results show that human serum albumin plus DHA exerts a far greater neuroprotection than that of albumin alone (16). Using rats subjected to 2 hours of middle cerebral artery occlusion followed by reperfusion, we have seen that intravenous administration of human serum albumin plus DHA greatly decreases cerebral damage, reduces edema, and increases the synthesis of NPD1 on the ipsilateral side (16). The implications of these studies are that human serum albumin plus DHA may be potentially useful as a therapeutic approach in stroke, traumatic head injury, spinal cord injury, related trauma conditions, and other pathologies requiring neuroprotection. Insofar as the mechanism of protections by human serum albumin-DHA, in addition to its use as a precursor for NPD1 synthesis, the fatty acid may be utilized for the replenishment of DHA in neural membrane phospholipids, which are degraded by ischemia-reperfusion (9, 27, 64).

These findings have several implications related to both the understanding of how brain modulates inflammatory injury responses, and to designing new experimental therapeutics for neurologic diseases. Therefore we have demonstrated novel DHA-signaling pathways that may lead to answers to clinically important questions regarding undiscovered mechanisms in stroke, traumatic head injury, spinal cord injury, and other diseases that involve a neuroinflammatory component. The potent bioactivity of NPD1 suggests the existence of a poten-

tially important target for therapeutic, neuroprotective interventions in these diseases.

CONCLUSIONS

NPD1, a DHA-derived mediator endogenously synthesized by brain as well as by neuroepithelium-derived RPE cells, is a modulator of signaling pathways that promote cell survival. One target of this mediator is the Bcl-2 family of proteins, a premitochondrial apoptotic signaling event under conditions of oxidative stress. As a consequence, downstream signaling that includes effector caspase-3 activation and DNA degradation is attenuated. NPD1 also potently counteracted cytokine-triggered pro-inflammatory COX-2 gene induction, another factor in cell damage. In ischemia-reperfusion-injured hippocampus and in neural progenitor cells stimulated by IL-1β, COX-2 expression seems to be related to NF-κB activation. NPD1 inhibits NF-κB and COX-2 induction under those conditions (34). Therefore, a similar regulatory mechanism may operate in RPE cells, since NPD1 down-regulates cytokine-mediated NF-κB activation. Pro-inflammatory injury of the RPE is involved in pathoangiogenesis and proliferative vitreoretinopathy, which occur in several diseases, including diabetic retinopathy. On the other hand, NPD1 neuroprotective bioactivity in brain ischemia-reperfusion includes decreased infarct size and inhibition of polymorphonuclear leukocyte infiltration (34). DHA promoted strong cytoprotection in RPE cells in culture confronted with oxidative stress. In vivo the active DHA supply to brain and retina from the liver through the blood stream is necessary for cell development and function, and may play a critical role in conditions where, due to enhanced oxidative stress, the polyunsaturated fatty acyl chains of membrane phospholipids are decreased as a consequence of lipid peroxidation, as occurs in aging, retinal degenerations, and neurodegenerations such as Alzheimer disease (43, 56). In ischemia, neurotrauma, and seizures, there is also loss of brain DHA due to enhanced phospholipase A_2-activated DHA-containing phospholipids breakdown (9, 27, 64). It is conceivable that DHA gives rise to a wide variety of bioactive mediators. Such a cascade is already limited by the presence of additional potentially specific and potent mediators as have been identified during brain ischemia-reperfusion damage (34). Further understanding of the signals that modulate synthesis of NPD1, and of other DHA-derived mediators, may be of therapeutic value for stroke, neurotrauma, and neurodegenerative diseases. Moreover, selective DHA-delivery systems to the retina and brain will be useful. NPD1 and its cellular target(s) may also allow the design of novel therapeutic approaches to manage RPE and brain cytoprotection and in turn enhance neural cell survival in several of these diseases.

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