Survival signalling in Alzheimer’s disease

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Abstract
Significant advancements in our understanding of cell-survival signalling in AD (Alzheimer’s disease) stem from recent investigations into the metabolism, trafficking and fate of the essential ω-3 fatty acid DHA (docosahexaenoic acid) (C22:6, n = 3). Brain synaptic terminals and neuronal plasma membranes are highly enriched in DHA, and deficiencies in this polyunsaturated fatty acid are characteristic of AD-affected brain. Oxidative stress, targeting phospholipids containing DHA, and age-related DHA depletion are associated with the progressive erosion of normal cognitive function in AD. Current studies support the idea that DHA itself and novel DHA-derived neural synapse- and membrane-derived lipid messengers have considerable potential to modulate cell survival signalling in stressed cultured neural cell models in vitro and in mammalian models of learning, memory and AD in vivo. Key players in this intrinsic rescue system include the α-secretase-processed neurotrophin sAPPα (soluble APPα (amyloid precursor protein α)) peptide, the DHA-derived 10,17-docosatriene NPD1 (neuroprotectin D1), a tandem brain cytosolic phospholipase A2 and 15-lipoxygenase enzymatic system that biosynthesizes NPD1, and a small family of anti-apoptotic neuroprotective genes that encode Bcl-2, Bcl-XL and Bfl-1 (A1). This paper reviews current ideas regarding DHA and the oxygenated DHA derivative NPD1, intrinsically triggered biolipid neuroprotectants that along with their associated rescue pathways, contribute to life-or-death decisions of brain cells during homeostasis, aging and neurodegenerative disease.

Key words: Alzheimer’s disease, amyloid β-peptide (Aβ), cytotoxic phospholipase A2, docosahexaenoic acid (DHA), neuron survival, soluble amyloid precursor protein (sAPP).
Abbreviations used: AD, Alzheimer’s disease; Aβ, amyloid β-peptide; ApoE4, ApoE4 -apolipoprotein E4 allele that functions in maintaining lipid homeostasis, shuttling lipids throughout the CNS (central nervous system), repairing injured neurons, maintaining synaptodendritic connections and scavenging neuronal toxins [4,5].

Introduction
AD (Alzheimer’s disease) represents a unique dysfunction and deterioration of the evolutionarily higher structures of the human association neocortex and limbic system. Lack of adequate animal models has been a serious impediment to a clearer understanding of the basic AD processes, although transgenic animals, such as those overexpressing βAPP (β-amyloid precursor protein) and the family of secretases that process them, and others harbouring defective human AD genes and stressed, aging human neural cells in primary culture have provided valuable clues. Originally described 100 years ago by the German neuropathologists Emil Kraepelin, Franz Nissl and Alois Alzheimer, AD is characterized as an initially insidious and progressive neurodegenerative affliction culminating in massive neuronal cell loss in the association, hippocampus and limbic regions of the human brain. Specifically, progressive deterioration and knock out of the large pyramidal neurons of layers 3 and 5 of the association neocortex, and progressive loss of synaptic proteins and contacts and diminution of inter-neuronal signalling, are thought to be the clinical basis for the advancement of cognitive decline. Misfolded proteins, protein clumping and aggregation, the inability to catabolize insoluble, disease-related inclusions, even from their monomeric forms, and brain-specific immune, inflammatory and oxidative consequences due to accumulated AD lesions are a constant and recurrent theme in AD neuropathology [1–6]. Prominent among these misfolded proteins are the insoluble, pro-oxidative, pro-inflammatory aggregates of small Aβ (amyloid β-peptide) proteins, 37–42-amino-acid peptides generated by the tandem β,γ-secretase cleavage of βAPP. Notably, this Aβ-generating amyloidogenic pathway shunts another important non-amyloidogenic α-secretase pathway that normally generates a secreted 612-amino-acid, neurotrophic and synaptotrophic isoform of amyloid precursor protein, the sAPPα [soluble APPα (amyloid precursor protein α)] ([1–6]; see below). Besides excessive Aβ peptide production and depleted βAPP membrane stores due to increased secretase-mediated processing, advancing age, hypoxic and ischaemia episodes in the cerebral vasculature, head injury, age-related oxidative stress, lipid mis-metabolism and brain inflammation are other risk factors that can either lower the age of AD onset or accelerate AD progression. Several additional independent risk factors for AD involve excessive or unusual cholesterol and cholesterol oxide (such as 24S-hydroxycholesterol) trafficking and the presence of the ApoE4 (apolipoprotein E4) allele that functions in maintaining lipid homeostasis, shuttling lipids throughout the CNS (central nervous system), repairing injured neurons, maintaining synaptodendritic connections and scavenging neuronal toxins [4,5].
While researchers and pharmaceutical companies alike have directed a great deal of time and effort towards developing NSAID (non-steroidal anti-inflammatory drug) and statin-based therapies and other approaches to neutralize neuroinflammation and lower cholesterol and related brain lipids [4–8] and alternative strategies such as employing antibodies to modulate Aβ deposition and its affects [9–11], it appears that a great deal of therapeutic benefit for AD can be gained by approaches using lifestyle changes. Diets enriched in antioxidants and ω-3 fatty acids may bolster the synthesis of NPD1 (neuroprotectin D1) [14,50].

Role of sAPPα in AD

Our understanding of the function of βAPP remains incomplete; however, this type 1 transmembrane glycoprotein is implicated in a variety of neuron-essential functions that include neurite outgrowth, neuronal adhesion, intercellular contact and axonogenesis [18–21]. βAPP forms part of a multimeric, multichannelled, ‘γ-secretase complex’ of the neural cell plasma membrane, consisting of γ-secretase, presenilin, nicastrin, presenilin enhancer 2 homologue and anterior pharynx defective 1 homologue that are integral, amphiphatic components of cholesterol-rich lipid raft domains [1,3,16]. This high-molecular-mass, flattened-heart-shaped integral membrane complex, along with other membrane- associated secretase enzymes, gives rise to amyloidogenic Aβ peptides 37–42 amino acids in length (Aβ37–Aβ42), as well as an α-secretase-processed 6120-amino-acid neurotrophic sAPPα [16,17]. As a secretion product of the non-amyloidogenic cleavage of βAPP, sAPPα exhibits structural and functional similarities to other growth factors, such as epidermal growth factor, and has itself multiple neuroprotective and neurotrophic functions [18]. These include increasing the in vitro proliferation of embryonic neural stem cells [18], the promotion of long-term survival of neurons in culture, in part by activating the phosphoinositide 3-kinase signalling pathway that increases the expression of anti-apoptotic members of the Bcl-2 gene family [19], and also stabilization of intracellular Ca2+ concentrations, protecting the neural cytoskeleton from structural collapse and the inhibition of intracellular pro-apoptotic signalling by Bad (Bcl-2/Bcl-XL-antagonist, causing cell death), caspase 3 and caspase 9 [18–20]. Interestingly, cholesterol over-abundance in lipid raft harbours the integral ‘γ secretase complex’ appears to favour formation of the more neurotoxic amyloidogenic forms of Aβ peptide [4,8]. Conversely, enhanced DHA availability to cultured brain cells contributes to both increased formation of sAPPα and the mediator NPD1 that promote neuroprotection [14,18,21]. Indeed the health benefits of DHA in homoeostatic brain functions that involve learning and memory, in the protection, regeneration and repair of neurons, in reducing the effects of neuronal injury, in the improvement of functional outcome after brain injury and in neutralizing the effects of Aβ-mediated neurotoxicity are considerable [12,13,15,21–30].

DHA

The ω-3 fatty acid DHA is a dietary essential and a major component of phosphoacylglycerols in the neocortical grey matter of mammalian brain and the retinal pigment epithelium [21–23]. Studies of plasma membrane synaptosomes isolated from grey matter contain DHA selectively esterified to phosphatidylethanolamine and phosphatidylserine, and during brain development, substantial amounts of DHA are especially critical to ensure neurogenesis and synaptogenesis in both the brain and retina [23–25]. In contrast with elevated cholesterol, increases of DHA in neural membranes enhance plasma membrane fluidity and contribute to dynamic lipid membrane substructures [21,24]. In fact, neural membranes have been classified as having fluidities based on their polyunsaturated acyl chains in phospholipids that are mainly DHA. Membrane fluidity-modulated catalysis in lipid raft microdomains may be enhanced when DHA is present, but this is entirely dependent on the nature of the catalytic reaction [23–27]. In vitro and in vivo studies indicate that conditioned medium in cell cultures, or diets enriched in DHA (docosahexaenoic acid) in animal studies, decreases Aβ secretion from brain cells and decreases brain Aβ peptide burden and its toxic effects [26,27], improves learning ability in Aβ-infused rats [12,28], protects from dendritotoxic pathology in transgenic AD mouse models [29], prevents neuronal apoptosis induced by soluble Aβ peptides [30], increases synaptic protein and phospholipid densities [13,15] and inhibits degradative endopeptidase activities [31]. Conversely, depletion of DHA in transgenic mouse models of AD activates caspases, promotes apoptosis and decreases NMDA (N-methyl-D-aspartate) receptor densities [32]. While DHA is a prime lipid peroxidation target in oxidative injury, and markers for lipid peroxidation are increased in AD brain [33,34], DHA concentrations are decreased in the brain lipid pools of AD patients [35–37]. Indeed dietary supplementation using DHA may be a useful therapy for multiple neuro-psychiatric disorders that include AD, and part of these neuroprotective effects may be mediated through the generation of novel oxygenated DHA mediators such as NPD1 [13,38,50].

DHA-derived 10,17S-DHA NPD1

NPD1 is a novel, stereospecific member of a family of anti-inflammatory neuroprotective bioactive lipids generated from DHA and is the first oxygenated DHA derivative [41,44,45,47,49,50]. As mentioned previously, DHA has been shown to down-regulate Aβ peptide secretion from aged and cytokine-stressed human neural cells in primary culture, thus lowering Aβ peptide’s potential neurotoxic paracrine, aggregative and pro-inflammatory effects [4,14]. This neurotrophic effect is accompanied by enhanced biosynthesis of NPD1, a novel oxygenated 10,17S-DHA formed enzymatically from endogenous membrane phospholipid DHA stores. Both DHA and NPD1 levels are reduced in CA1 hippocampal regions from AD patients [14,47]. The expression of key enzymes in DHA mobilization
A wealth of potential pro-apoptotic or pro-survival signals is stored in membrane integral protein and esterified lipid reservoirs

Two key players in cell survival signalling in AD are shown, namely βAPP and DHA. βAPP holoprotein is highly enriched in neuronal membranes and via tandem β,γ-secretase activity gives rise to amyloidogenic Aβ40 and Aβ42 peptides. These in turn self-aggregate and promote proinflammatory gene signalling that gives rise to oxidative stress, apoptosis and neural cell demise. On the other hand, via the α-secretase pathway, a secreted non-amyloidogenic neurotrophic sAPPα peptide is generated whose bioactivity supports neurotogenesis and cell survival. DHA is highly enriched as an acyl side chain of brain and retinal membrane phospholipids, suggesting its intrinsic importance as an essential component of brain and retinal function. A tandem PLA2–15-LOX membrane-associated enzymatic system liberates esterified DHA, whereupon it is liberated and oxygenated to form 10,17-DHA (NPD1). Whether other phospholipases or lipoxygenases contribute to the NPD1 biosynthetic pathway is not known and is currently under active investigation. sAPPα stimulates NPD1 biosynthesis and NPD1 possesses multiple neuroprotective activities against Aβ40 and Aβ42 peptides, the more neurotoxic pro-apoptotic forms of βAPP cleavage products. DHA and NPD1 in particular exert neurotrophic effects via the repression of pro-inflammatory gene expression, in part via inhibition of NF-κB (nuclear factor κB)-, AP1 (activator protein 1)-, HIF-1 (hypoxia-inducible factor-1)- and STAT-1 (signal transducer and activator of transcription 1)-mediated transcription factor signalling enriched in the promoters of a family of pro-inflammatory genes [4,35,39,45,57–59,62]. DHA and NPD1 also repress neural apoptosis and promote the expression of anti-apoptotic genes encoding Bcl-2, Bcl-XL and Bfl-1(A1), while repressing the expression of the pro-apoptotic genes Bax, Bad, Bid and Bik [13,45]. These recent findings expand our understanding of how the CNS counteracts oxidative disturbances and redox imbalances, mitochondrial dysfunction, pro-inflammatory gene expression and their consequences. Biologically important control gates for neuronal survival at the level of α-, β- and γ-secretases, PLA2 and 15-LOX activities may be profitably explored to modulate various aspects of brain cell injury. Indeed the specificity and potency of NPD1 indicate a powerful endogenous target for the therapeutic intervention of pro-inflammatory and degenerative brain disease [14,50].

**Figure 1** | A wealth of potential pro-apoptotic or pro-survival signals is stored in membrane integral protein and esterified lipid reservoirs

and NPD1 biosynthesis, cPLA2 [cytosolic PLA2 (phospholipase A2)] and 15-LOX (15-lipoxygenase), was found to be increased and decreased respectively in short post-mortem AD brain [13]. Activation of pro-apoptotic and pro-inflammatory genes in Aβ42 (Aβ 42-amino-acid variant)-stressed HN cells was found to be repressed by both DHA and NPD1, with concomitant up-regulation of the Bcl-2 anti-apoptotic proteins Bcl-2, Bcl-XL, and Bfl-1(A1) (Figure 1). The neurotrophin sAPPα was found to stimulate NPD1 production [13,50]. NPD1 was found to inhibit Aβ42-induced HN cell apoptosis. These results indicate that NPD1 mediates induction of both anti-apoptotic and neuroprotective gene-expression programmes that down-regulate Aβ secretion and modulate intrinsic anti-inflammatory signals that promote neural cell survival in AD brain [41–50]. The stereospecificity and potency of NPD1 indicate a potential target for therapeutic intervention for stroke, age-related macular degeneration, spinal cord injury and other neuroinflammatory disorders.

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or neurodegenerative diseases. As the membrane-associated brain cPLA2 and 15-LOX activities in tandem appear to be intrinsic to the oxygenation of DHA to yield NPD1, their neurochemistry will be further reviewed below.

Brain PLA2 (cPLA2) and 15-LOX

The amount of free (non-esterified) DHA (and arachidonic acid) is very small under basal, homoeostatic conditions in the brain; however, these amounts are dramatically increased after synaptic activity, ischaemia, hypoxia and neurodegenerative events [51]. As the DHA pool size is tightly controlled by the action of phospholipase, the regulation of phospholipases that release DHA from membrane reservoirs is critical in the formation of bioactive docosanoids (Figure 1). Multiple isoforms of PLA2 in the mammalian brain, both cytosol-enriched and secreted, are involved in a complex network of signalling pathways that link receptors and inflammatory cytokines to the release of DHA, arachidonic acid and the biosynthesis of eicosanoids [51,53–57]. Human cPLA2 (GenBank® accession no. D38177) gene encodes an inducible 85-kDa calcium-dependent carboxyl esterase that excises and mobilizes free DHA (and arachidonic acid) from brain plasma-membrane phospholipid stores [3,4]. This key glycerophospholipid esterase hydrolyses sn-2-acyl ester bonds, generating arachidonic acid, driving the arachidonic acid cascade and liberating DHA [51–55]. Interestingly, cPLA2 and the endoperoxide synthetase COX-2 (cyclooxygenase-2) often operate in tandem on the breakdown of membrane glycerophospholipid stores, generating bioactive lipids that further promote endothelial-cell proliferation and angiogenesis [55–58]. cPLA2 is rate-limiting for arachidonic acid release and the generation of prostaglandins, leukotrienes, hydroxyeicosatetraenoic acids and related bioactive lipids via the arachidonic acid cascade. These play determinant roles in cellular development, proliferation, differentiation, vascular homeostasis and the immune response; however, high levels of lipid mediators generated by cPLA2 are also involved in brain pathologies associated with neural inflammation, oxidative stress, neural cell injury, dysfunction and neural cell death. The expression of cPLA2 is rapidly activated as a response to hypoxia in a number of different human cell lines, including those of the retinal and umbilical vein endothelium and in human brain cells, and cPLA2 mRNA is up-regulated in AD and in related neurodegenerative disorders associated with a cerebrovascular component, ischaemia and hypoxia [57–62]. Interestingly, the human genes encoding both cPLA2 and COX-2, which acts on the arachidonic acid product of cPLA2, have been previously localized to this same chromosomal region, raising the possibility of co-ordinate gene regulation [63]. The enzymatic system responsible for the second step of NPD1 syntheses encodes a membrane-associated (15-LOX) that generates the 10,17-H(p)DHA NPD1 via 17S-H(p)DHA and a 16(17)-epoxide intermediate (Figure 1) [50,64–66]. Whether other phospholipase or lipoxygenase systems are involved in ancillary NPD1 production is currently not well understood and is an active area of study. Nanomolar quantities of NPD1 have been shown to be a potent inhibitor of pro-inflammatory gene expression and a repressor of COX-2, IL-1β (interleukin 1β), CEX-1 (chemokine exodus protein 1), B94, caspase 3 and the pro-apoptotic proteins Bax, Bad, Bid and Bik. In contrast, NPD1 appears to be a major positive inducer of the neuroprotective Bcl-2 proteins Bcl-XL and Bfl-1(A1) proteins [13,50].

The anti-apoptotic gene family encoding Bcl-2, Bcl-XL and Bfl-1(A1)

Lastly, programmed cell death or apoptosis, the critical process for normal embryonic development, homoeostasis and neurodegeneration, involves multiple signalling pathways and crucial modulators of cell survival, the Bcl-2 family of proteins. These cell-fate decision proteins through their heterodimerization either support or repress mitochondrial apoptotic pathways and neural cell death. Caspase 3, an apoptotic effector acting downstream of the pro-apoptotic and anti-apoptotic Bcl-2 proteins, is activated as a consequence of mitochondrial cytochrome c release into the cytoplasm and activation of the apoptosome [42,45]. Catabolism of endogenous substrates by caspase 3 is enhanced by inflammation and oxidative stress. The AD Aβ42 peptide induces cell death in human neuroblastoma cells and caspase 3 activation initially via a Bax/Bcl-2 ratio increase [67], and Bcl-2 and Bax induction is related to hyperphosphorylation of tau and neuronal death induced by okadaic acid in rat brain [68,69]. Wide-spectrum gene expression studies show similar depletion of the anti-apoptotic members of the Bcl-2 gene family in AD hippocampus and superior temporal lobe neocortex, and a shift in expression towards the more pro-apoptotic Bcl-2 family species Bax, Bad, Bid and Bik [39,42,44,57,59]. Depletion of these endogenous neuroprotective signals directly contributes to the widespread apoptotic-mediated loss of neurons characteristic of AD brain.

Conclusions

A non-homoeostatic balance of neurotoxic and neurotrophic signalling influences the ultimate fate of neocortical and limbic system pyramidal neurons in AD. Whether intrinsic ‘rescue’ mechanisms are triggered as the result of age- or pathology-related gene mis-regulation, via signalling through these neurotoxic gene products, or are attempts by pathology-stressed neural cells to alter or reverse their cellular fate is not known. However, mobilization of neural molecular survival signals such as those that direct angiogenesis, neurogenesis, the heat-shock response and intrinsic neuroprotective signalling directed against Aβ toxicity suggests the latter. Exogenously supplied DHA has been shown to attenuate Aβ secretion and neutralize Aβ neurotoxicity in both neural cellular and animal models. When supplied with exogenous DHA, cytokine-stressed human neural cells direct the
formation of NPD1 that in turn reduces Aβ peptide secretion and induces both anti-apoptotic and neuroprotective gene-expression programmes (Figure 1) [14,43–45,47,50]. Another important in vivo action of NPD1 may be to neutralize Aβ-mediated neurotoxicity by redirecting βAPP processing and catabolism into favoured production of the non-amyloidogenic, NPD1-inducing sAPPα peptide, and thus promote survival in inflammation- or oxidation-stressed brain cells. The biological actions of DHA and NPD1 on the α-, β- and γ-secretase activities that differentially process membrane-bound βAPP are not known. Lifestyle changes to diets enriched in antioxidants and ω-3 fatty acids such as DHA might be expected to support the recruitment of potent endogenous neuroprotective survival pathways, and thus represents a highly desired approach and strategic prophylactic therapy for AD intervention. Future research should determine whether NPD1 analogues may be the basis for novel therapeutic approaches for neurodegenerative drugs. In addition, agonists for the formation of endogenous DHA-derived neural survival signal NPD1, and the endogenous phospholipases and lipoxygenases that biosynthesize NPD1, can be more directly manipulated to ameliorate the progressive course of AD and related neurodegenerative disorders.

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References


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