SECRETORY PHOSPHOLIPASE A2-IIA IN ALZHEIMER'S DISEASE AND INFLAMMATORY RESPONSES IN ASTROCYTES

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

SECRETORY PHOSPHOLIPASE A2-IIA IN ALZHEIMER'S DISEASE AND INFLAMMATORY RESPONSES IN ASTROCYTES

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A candidate for the degree of Doctor of Philosophy

And hereby certify that in their opinion it is worthy of acceptance.

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LIST OF ABBREVIATIONS

5-LOX	5-lipoxygenase
AA	arachidonic acid
AD	Alzheimer's disease
APP	amyloid-β precursor protein
cAMP	cyclic adenosine monophosphate
C/EBP	CCAAT enhancer-binding protein
CNS	central nervous system
COX-2	cyclooxygenase-2
DNA	deoxyribonucleic acid
EGCG	epigallocatechin gallate
ERK	extracellular signal-regulated kinase
FAD	familial Alzheimer's disease
FBS	fetal bovine serum
HSPG	heparan sulfate proteoglycans
ICAM-1	intercellular adhesion molecule 1
iNOS	inducible nitric oxide synthase
IL-1β	interleukin-1β
IL-6	interleukin-6
IL-8	interleukin-8
MAPK	mitogen-activated protein kinase
NADPH	nicotinamide adenine dinucleotide phosphate
ND	non-demented
NFκB	nuclear factor κB
NO	nitric oxide
NOX	NADPH oxidase
pERK	phosphorylated extracellular signal-regulated kinase
PI3K	phosphatidylinositol 3-kinase
PIP3	phosphatidylinositol (3,4,5)-trisphosphate
PKC	protein kinase C
PGE2	prostaglandin E2
PGD2	prostaglandin D2
ROS	reactive oxygen species
TNF	tumor necrosis factor
TNF-α	tumor necrosis factor-α

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ABSTRACT

Alzheimer's disease (AD) is a progressive, fatal neurodegenerative disease and is the most common form of dementia. AD pathology includes neurofibrillary tangles, amyloid plaques, chronic inflammation, and oxidative stress. Secretory phospholipase A2-IIA (sPLA2-IIA) is an inflammatory protein known to have a role in the pathogenesis of multiple inflammatory diseases and is implicated in several neurodegenerative diseases. In AD, astrocytes become reactive and have increased expression of inflammatory cytokines such as IL-1β and TNF-α, and undergo increased oxidative stress. NADPH oxidase is one of the major enzymatic sources of reactive oxygen species in the central nervous system; activation of this enzyme may contribute to increased oxidative stress in cells of AD brains, including astrocytes. The expression and localization of sPLA2-IIA in human AD brains has not been studied in detail. In these studies, we show that sPLA2-IIA mRNA is up-regulated in AD brains (compared to non-demented elderly brains) and sPLA2-IIA immunoreactivity is increased in AD astrocytes. To further elucidate involvement of oxidative pathways in induction of sPLA2-IIA mRNA and protein by pro-inflammatory cytokines, we performed in vitro studies with immortalized astrocytes (DITNC). These studies demonstrated the involvement of PI-3 kinase and ERK1/2, but not p38 MAPK, in the cytokine-induced sPLA2-IIA expression

in astrocytes. Furthermore, inhibition of sPLA2-IIA mRNA expression by apocynin, a known NADPH oxidase inhibitor, and botanical antioxidants including resveratrol and epigallocatechin gallate, suggests the involvement of oxidative pathways, possibly the NADPH oxidase pathway. These results, taken together, identify sPLA2-IIA as an inflammatory factor for Alzheimer's disease, and support the involvement of NADPH oxidase in the cytokine induction of sPLA2-IIA in astrocytes and the possibility of using botanical antioxidants to ameliorate the inflammatory response in these cells.

Chapter 1

INTRODUCTION

Phospholipase A2

Phospholipases A2 (PLA2s) are essential enzymes for maintenance of cell membrane phospholipids. The PLA2 family is grossly separated into three groups; namely, group IV calcium-dependent cytosolic PLA2 (cPLA2), group VI calcium-independent PLA2 (iPLA2) and group II secretory PLA2 (sPLA2) (Murakami and Kudo, 2002a; Burke and Dennis, 2009b). Despite being very different enzymes, they all catalyze the hydrolysis of the *sn*-2 acyl ester linkage of phospholipids, generating free fatty acids and lysophospholipids. The physiological functions of PLA2s include phospholipid remodeling, exocytosis, and neurotransmitter release (Valentin et al., 1999; Bazan, 2003). PLA2s also play a key role in the production of lipid mediators and the mediation of cellular responses. Therefore, it is reasonable to assume that different PLA2s undergo different regulation and dysregulation may be an underlying cause of various disease processes (Farooqui et al., 1997a; Sun et al., 2004; Farooqui and Horrocks, 2006). Previous studies had focused on the Group IV cPLA2 because this enzyme is linked to a number of receptor-mediated cell signaling pathways (Xu et al., 2002; Hirabayashi et al., 2004). Although Group II sPLA2s are regarded as enzymes involved in inflammatory pathways (Fuentes et al., 2002; Lambeau and Gelb, 2008), little is known about their role in the central nervous system (CNS). The focus of this research project is to investigate the regulation of sPLA2-IIA, one of the isoforms of sPLA2, in the brain and to elucidate its role in neuroinflammatory processes.

Secretory PLA2s

The mammalian sPLA2s are calcium-dependent enzymes with small molecular weight (~14 kDa), several disulfide bridges (4-8), and a strongly conserved active site. There are more than 10 isoforms of sPLA2s, including groups IB, IIA, IIC, IID, IIE, IIF, III, V, X, and XII. The naming structure is based on the disulfide bond pattern and the order of their discovery (Lambeau and Gelb, 2008).

While the mouse genome contains ten sPLA2 genes, the human genome contains only nine, because the group IIC sPLA2 only exists as a pseudogene in humans (Tischfield et al., 1996). Most sPLA2s have an N-terminal signal peptide and are known to be secreted from their native tissues. Group IB is secreted as a zymogen into the gastrointestinal tract. Upon activation by removal of the propeptide by trypsin, it is recognized as an enzyme in pancreatic juice (Verheij et al., 1981). An unknown protease removes the propeptide from sPLA2 X, and the propeptide-free mature protein is secreted into the culture medium of transfected cells (Hanasaki et al., 1999).

Mammalian sPLA2s have a close relation to the snake venom sPLA2s. Instead of using a classical acyl enzyme intermediate like serine proteases, secretory PLA2 enzymes utilize the catalytic site His and the assistance of an Asp to polarize a bound H_20, which then attacks the carbonyl group. sPLA2s are calcium-dependent, and the Ca^{2+} ion is bound in the conserved Ca^{2+} binding loop to provide stabilization for the enzyme transition state (Verheij et al., 1981; Dennis, 1994).

sPLA2 and eicosanoid synthesis

The non-pancreatic sPLA2s are involved in hydrolysis of intracellular membrane phospholipids leading to release of arachidonic acid (AA), which serves as substrate for synthesis of a number of eicosanoids, including leukotrienes and prostaglandins. In neurons, AA has been shown to incorporate into synaptic vesicles and mediate neurotransmitter release. These fatty acids may alter membrane curvature and promote membrane fusion through interaction with lysophospholipids on the cell membrane, and thus participate in exocytosis and neurotransmitter release (Wei et al., 2003). Many studies on eicosanoid synthesis show cooperation between sPLA2 and either cPLA2 (group IVA) or iPLA2 (group VIB PLA2). The relationship between sPLA2 and cPLA2-alpha is particularly relevant because cPLA2-alpha is known to target phospholipid substrates for release of AA (Ghosh et al., 2006). Overexpression of sPLA2-IIA (mouse) in HEK293 cells was shown to cause an increase in AA release and prostaglandin E2 (PGE2) production (Murakami et al., 1998; Mounier et al., 2004). Other studies also show the cross-talk of cPLA2-alpha with sPLA2-IIA for PGE2 production (Akiba et al., 2001; Han et al., 2003; Mounier et al., 2004; Ni et al., 2006).

sPLA2 receptors

Besides the action of sPLA2-IIA in releasing lipid mediators for eicosanoid synthesis, sPLA2s may also behave as ligands for receptors. This denotes that sPLA2s may have a physiological function outside of their catalytic activity. Both M-type (muscular) and N-type (neuronal) receptors have been identified, although no exogenous ligand has been identified for the N-type sPLA2 receptor (Lambeau and Lazdunski, 1999). N-type receptors are highly expressed in mammalian brain membranes and have

been shown to bind neurotoxic sPLA2s with high affinity. M-type receptors contain a carbohydrate recognition domain and are part of the C-type lectins superfamily. M-type receptors can be internalized, possibly to inhibit sPLA2 activity through degradation. This control of sPLA2 activity is confirmed by the role of other C-type lectin proteins that can bind and inhibit sPLA2s as well. Several mammalian sPLA2s (including IIA and V) have shown to bind to various types of heparan sulfate proteoglycans (Hurt-Camejo et al., 2001; Boilard et al., 2003).

sPLA2s in the central nervous system

Little is known about sPLA2s in the central nervous system (CNS). The following is a brief review of the characteristic traits and functions of each of the ten groups of sPLA2s. Major focus will be placed on the role of sPLA2-IIA in the CNS.

sPLA2-1B: sPLA2-1B is included in the pancreatic enzymes, as it is secreted by the pancreas and found abundantly in the digestive tract, where it hydrolyzes phospholipids in the intestinal lumen for aiding dietary lipid absorption (Carey et al., 1983). The mammalian presence of sPLA2-1B in the brain appears to be mainly neuronal, occurring mostly in the cerebral cortex and the hippocampus. It is suggested that sPLA2-1B may be a neuronal intercellular signaling modulator (Kolko et al., 2005). sPLA2-1B has also been found to act as an endogenous PLA2 receptor ligand leading to cell proliferation, cell migration, and lipid mediator production (Lambeau and Lazdunski, 1999; Hanasaki and Arita, 2002). Group 1B can promote neuronal cell death based on binding with the M-type sPLA2 receptor (Yagami et al., 2002b).

sPLA2-IIA

sPLA2-IIA was first identified in atherosclerotic lesions, and is a known inflammatory marker in atherosclerosis. Due to its involvement in inflammatory processes in the peripheral systems, the IIA isoform is the most studied of the group II sPLA2s (Murakami et al., 1997; Murakami and Kudo, 2002a).

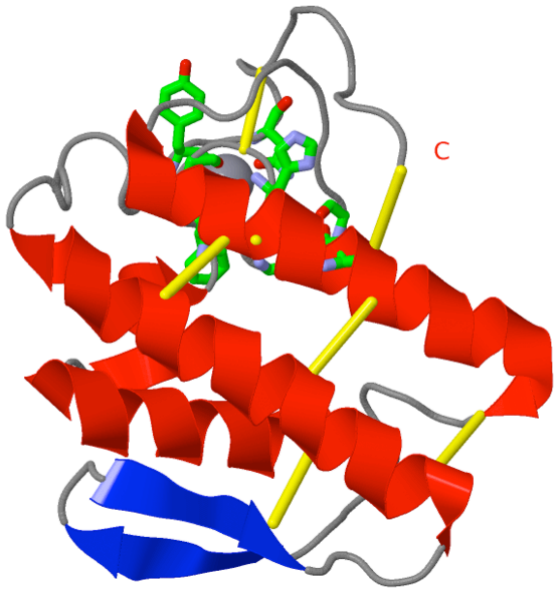

Figure 1.1 – Structure of human secretory phospholipase A2 IIA from inflammatory exudate. http://www.proteopedia.org/wiki/index.php/1pod

Structure of sPLA2-IIA

The human group IIA gene is tightly linked to sPLA2 V and IIC pseudogenes on chromosome 1p34-p36.1, and contains three introns. The gene product is expressed as a prepeptide, with the first 20 amino acids most likely acting as a signal peptide until being cleaved (Tischfield, 1997). The protein itself contains 6 conserved disulfide bonds, and

has a C-terminal extension of six amino acids terminating in a Cys near the catalytic site (denoted with C in figure 1.1). In figure 1.1, you can see the six conserved disulfide bonds (in yellow). The active site of PLA2s contains a catalytic triad of Asp-99, His-48, and a water molecule, which sits between the two parallel helices. Its mechanism involves binding a single lipid molecule in the active site at the lipid-water interfacial binding site and subsequent hydrolysis (Scott et al., 1990; Kumar et al., 1994).

Regulation of sPLA2-IIA

Most group II genes are clustered in chromosome 1 (Suzuki et al., 2000). However, sPLA2-IIA is not constitutively expressed under basal conditions in most cell types, but is induced in response to inflammatory stimuli. Factors that affect the regulation of sPLA2-IIA include cytokines, bacterial toxins, and growth factors. Although the effect on transcriptional regulation of sPLA2-IIA is cell- and species-specific, sPLA2-IIA gene expression is typically up-regulated by cytokines, including interleukin-1-beta (IL-1β), interleukin 6 (IL-6), and tumor necrosis factor alpha (TNF-α), and is down-regulated by growth factors (Andreani et al., 2000).

Transcriptional regulation of sPLA2-IIA has not been extensively studied; however, several factors have been identified in the regulation of sPLA2-IIA in various cell types and species. In humans and rats, C/EBP and NF-κB are considered to be the major transcription factors. In human hepatocytes, single stranded DNA binding proteins repress sPLA2-IIA expression. CTF/NF1 and SP1 may also bind to and regulate both the rat and human promoter, while peroxisome proliferators-activated receptors may be a

transcription factor in the rat promoter of vascular smooth muscle cells (Andreani et al., 2000).

Distribution of sPLA2-IIA

In the brain, sPLA2-IIA has a generally ubiquitous expression, and is found at high levels in inflamed tissues. Through studies of rat brain, sPLA2-IIA is known to be found in cortex, midbrain, spinal cord, and brain stem, with lower levels found in cerebellum, corpus striatum, olfactory bulb, hippocampal CA1-3, amygdala, basal forebrain, and cerebral cortex (Molloy et al., 1998; Svensson et al., 2005; Adibhatla et al., 2006). sPLA2-IIA is known to be associated with the endoplasmic reticulum (Bingham et al., 1999; Murakami et al., 1999) and is localized in the perinuclear region of Purkinje cells (Shirai and Ito, 2004).

Functions of sPLA2-IIA

sPLA2-IIA is involved in neuronal apoptosis, possibly through the metabolites of prostaglandin D2 (PGD2), the predominant prostaglandin in the mammalian brain. In the Yagami et al. study, stroke-induced cerebral ischemia activates inflammatory factors that induce sPLA2-IIA, which liberates AA and subsequently generates PGD2 and triggers apoptosis of neurons in the penumbra (Yagami et al., 2002a).

In the peripheral system, sPLA2-IIA has been implicated in the pathogenesis of several inflammatory and infectious diseases, including allergies, rheumatoid arthritis, and septic shock (Vadas et al., 1993; Jamal et al., 1998; Liu et al., 2007). Up-regulation of sPLA2-IIA has been shown in a number of cancer tissues (Kashiwagi et al., 1999;

Jiang et al., 2002). In the CNS, sPLA2-IIA has also been implicated in a number of neurodegenerative diseases including Alzheimer's disease (AD), multiple sclerosis, and stroke (Lin et al., 2004). Since AD brains are thought to experience chronic inflammation, which is marked by an increase in inflammatory factors such as cytokines, it is reasonable that the AD brain is associated with an increase in sPLA2-IIA. Indeed, a recent joint study by our laboratory and by Dr. L.F. Lue's laboratory demonstrated up-regulation of sPLA2-IIA mRNA and immunoreactivity in the AD brain as compared to non-demented controls (Moses et al., 2006) and Chapter 3). However, studies to further investigate the role of sPLA2-IIA in AD pathophysiology using transgenic mouse models have been hampered due to a frameshift mutation in exon 3 in the sPLA2 gene in the C57BL/6 strain, the most popular mouse strain for genetic modifications (Kennedy et al., 1995). Another hurdle for studies of sPLA2-IIA is the lack of specific antibodies. Nevertheless, increases in sPLA2-IIA mRNA, protein expression, and PLA2 activity in rat brain after cerebral ischemic stroke (Lin et al., 1996; Farbman et al., 2004; Adibhatla and Hatcher, 2007).

Inhibitors of sPLA2-IIA

Although over forty structural classes of sPLA2 inhibitors exist, the potency of the majority of sPLA2 inhibitors is not reliable and many studies use micromolar quantities to attain effects. Furthermore, although most inhibitors, such as varespladib (Indole), compound 2b (amide-based analog), Pyrazole-1, and YM-26734 are targeted to sPLA2-IIA, some also inhibit other sPLA2s, e.g., sPLA2-X (varespladib) or IID (Pyrazole 1) (Reid, 2005; Oslund et al., 2008; Burke and Dennis, 2009a).

sPLA2-IIC:sPLA2-IIC has been found in rat, mouse, and humans, but in humans sPLA2-IIC appears to be a non-functional pseudogene (Tischfield et al., 1996). A study looking at various rat tissue RNAs, and RNA from whole testis at different stages of development, concluded that group IIC may only be expressed at significant levels in testis and is developmentally regulated (Chen et al., 1997).

sPLA2-IID:Group IID is a heparin-binding sPLA2 with a close relation to sPLA2-IIA. Similar to sPLA2-IIA, sPLA2-IID increases stimulus-induced AA release (Murakami et al., 2002b). sPLA2-IID is suggested to have a role in endotoxic shock due to its enhanced expression in rat thymus and in sPLA2-IIA-deficient mice upon endotoxin challenge (Ishizaki et al., 1999). The regulatory mechanisms of sPLA2-IID differ from its closely related isozymes IIA and V, and also appear to be cell- and tissue-specific (Murakami et al., 2002b).

sPLA2-IIE: sPLA2-IIE shows the highest homology and has the most similar *in vitro* enzymatic properties with groups IIA and IID (Valentin et al., 1999; Suzuki et al., 2000). In mammalian cells, sPLA2-IIE can be induced in response to proinflammatory stimuli, and promotes stimulus-induced AA release and eicosanoid generation. With this ability, sPLA2-IIE can be a participant in inflammation involving mast cell-directed allergic reactions (Murakami et al., 2002a). Compared to group IIA, sPLA2-IIE has lower expression levels in physiological states. Unlike IIA and IID, which are more widespread among tissues, sPLA2-IIE transcripts were limited to the brain, heart, lung, and placenta

and elevated expression was found in alveolar macrophage-like cells in the lung of endotoxin-treated mice (Suzuki et al., 2000).

sPLA2-IIF: Group IIF sPLA2 is unique in its ability to traverse the plasma membrane of mammalian cells independent of heparan sulfate proteoglycan (HSPG) binding, which acts as a functional adaptor for other sPLA2-IIs. This is due to its unique structure including a long cluster of hydrophobic residues (proline-rich) near the C-terminal extension. sPLA2-IIF can enter the cell in an endocytosis-independent manner, but upon entering the cell no significant change in phospholipid hydrolysis is found. Overexpressed sPLA2-IIF in mammalian cells was shown to induce cellular AA and eicosanoid formation (Murakami et al., 2002c; Wijewickrama et al., 2006b).

sPLA2-III: Group III is unique among the other isozymes as it has both N-terminal and C-terminal domains with a central sPLA2 domain homologous to bee venom group III sPLA2. In mammalian sPLA2-III, the protein is processed to the mature and enzymatically active form consisting of just the central sPLA2 domain (Valentin et al., 2000; Murakami et al., 2003). sPLA2-III is found in both the peripheral and central nervous systems, in the vascular endothelium of various tissues, alveolar epithelium and macrophages, and in several types of cancer tissue (Murakami et al., 2005; Masuda et al., 2008; Mounier et al., 2008). With respect to its arachidonate-releasing function (from overexpression studies), sPLA2-III is more effective than group IIA, but less effective as compared to groups X and V (Murakami et al., 2003; Murakami et al., 2005). Similar to sPLA2-X, group III sPLA2 is shown to support neuronal outgrowth and survival through

its neuritogenic and neurotropic actions on neuronal cells and production of lysophosphatidylcholine (LPC; (Masuda et al., 2008).

sPLA2-V: sPLA2-V was first identified in the human brain, and is also known to be prevalent in the heart (Chen et al., 1994b, a). The transcellular activity of sPLA2-V is highly dependent on the presence of cell surface HSPGs of acceptor cells. A study showed that cells expressing endogenous sPLA2-V may function to trigger inflammatory cell activation, in particular, in the neighboring cells (Wijewickrama et al., 2006a). sPLA2-V also plays a role in lipid mediator production and phagocytosis in macrophages (Balboa et al., 1996; Satake et al., 2004; Balestrieri et al., 2006), in atherosclerosis development (Bostrom et al., 2007), and lung surfactant hydrolysis (Ohtsuki et al., 2006).

sPLA2-X: sPLA2-X has known expression in the human spleen, thymus, peripheral leukocytes, and brain, and appears to be involved in functions relating to immunity and inflammation (Cupillard et al., 1997). sPLA2-X is considered to be the most potent hydrolyser of the outer plasma membrane (Murakami et al., 1998; Hanasaki et al., 1999; Bezzine et al., 2000). Despite low levels of expression in peripheral neurons, sPLA2-X has a neuritogenic effect, promoting neuronal outgrowth and survival through production of LPC (Nakashima et al., 2004; Ikeno et al., 2005; Masuda et al., 2005; Masuda et al., 2008). sPLA2-X also plays a role in airway inflammation and remodeling induced by allergens (Henderson et al., 2007), and in myocardial ischemia/reperfusion injury (Fujioka et al., 2008).

sPLA2-XII: Group XII is more distantly related to the other sPLA2s. sPLA2-XII is known to be expressed in antigen-activated helper T cells in mice where it may participate in the immune response through eicosanoid generation and immediate second signals (Ho et al., 2001). Its activity, however, appears to be extremely low toward phosphatidylethanolamine and phosphatidylcholine, and Group XII is thought to not have sufficient catalytic activity to mobilize cellular AA (Murakami et al., 2003).

Neuroinflammation

Various types of insults to the CNS, such as infection, trauma, and stroke, are known to give a patterned response called neuroinflammation. Neuroinflammation is also part of the pathogenesis of many neurodegenerative diseases including Alzheimer's disease, Huntington's disease, multiple sclerosis, and Parkinson's disease (Nguyen et al., 2002). Hallmarks of neuroinflammation include microglia activation and increased release of inflammatory mediators (Shaftel et al., 2007). Microglia are the immune cells within the brain, and during inflammation they become over-stimulated with various cues and then produce cytotoxic factors including superoxide, nitric oxide, IL-1β, and TNF-α. At the point where microglia become too activated, they transform from being important for host defense to being neurotoxic (Polazzi and Contestabile, 2002; McGeer et al., 2005). Acute inflammation is the immediate and early response to tissue injury and, when left unresolved, will lead to chronic inflammation. In chronic inflammation, there is also an increase in tissue destruction by inflammatory cells and repair.

Neuronal cells are susceptible to damage due to the effects of reactive oxygen species (ROS) because of their high energy and oxygen consumption rate, abundance of

polyunsaturated fatty acids, low levels of antioxidants, high levels of transition metals that can catalyze reactions generating reactive hydroxyl radicals, and a reduced capability to regenerate (Zaleska and Floyd, 1985; Floyd and Carney, 1992; Floyd, 1999). Microglia and astrocytes can turn from being supportive cells to damaging cells generating harmful free radicals early in the inflammation process. Activated microglia contribute to inflammatory processes by producing neurotoxins such as quinolinic acid, superoxide anions, nitric oxide (NO), arachidonic acid, chemokines, proinflammatory cytokines, and excitotoxins like glutamate. Free radicals can cause oxidative damage to proteins, lipid bilayers, and even DNA through strand breaks, DNA-protein cross-linking, and base modifications (Nunomura et al., 1999; Reynolds et al., 2007).

Alzheimer's disease

Alzheimer's disease (AD) is the fourth leading cause of death in persons over the age of 65 years old and is the most common disease of all neurodegenerative diseases that cause dementia. Alzheimer's disease is currently incurable and existing treatments can only slow its progression. The physical progression of Alzheimer's disease involves the cognitive decline of the individual leading to clinical dementia. In terms of pathology, AD is suggested to be a form of neuroplasticity failure. Learning and memory depends on synaptic efficacy, but in AD there is a disruption in synaptic plasticity and neuronal impairment, the source of which can be pointed at a number of different molecular contributors. Neuropathologies of AD include senile plaques (amyloid-beta), neurofibrillary tangles (tau), oxidative stress, and inflammation (Schwab and McGeer, 2008; Gella and Durany, 2009).

Senile plaques are primarily composed of depositions of amyloid-beta (A-β) peptide. Amyloid precursor protein (APP) is a transmembrane protein, and can be cleaved by β-secretase (also called BACE1, β-site of APP cleaving enzyme) followed by a second cleavage from γ-secretase to produce mostly A-β(1-40) but it can also produce the A-β(1-42) cleaved fragment. A-β(1-42) is highly hydrophobic and leads to amyloid fibrillogenesis, which is implicated in the pathogenesis of AD. The fibrillar form of A-β is formed over many years, where these deposits can be compacted into mature senile plaques, becoming fibrillar and having a parallel beta-sheet conformation (Cerf et al., 2009). In this fibrillar form, A-β is neurotoxic and is thought to significantly contribute to the pathology of Alzheimer's disease (Halliday et al., 2000). The nonaggregated form of A-β, oligomeric A-β, is characterized by its anti-parallel beta-sheet structure (Cerf et al., 2009). Oligomeric A-β is also considered neurotoxic, arguably more than the fibrillar form of A-β (Dahlgren et al., 2002). Genetic studies of early-onset familial AD (FAD) give A-beta its strong correlation with AD with several FAD mutations being found in the amyloid precursor protein (APP) and the presenilin genes (Selkoe, 1996).

Neurofibrillary lesions made of hyperphosphorylated microtubule-associated protein tau is the other dominant pathology in Alzheimer's disease next to A-beta deposition. In AD, intracellular neurofibrillary lesions are associated with A-beta deposits, an occurrence also seen in Down's syndrome. Three main types of neurofibrillary lesions are localized in nerve cells, including neurofibrillary tangles in the cell body, neuropil threads in distal dendrites, and abnormal neuritis associated with neuritic (senile) plaques (Probst et al., 1991).

Cells in the CNS are susceptible to oxidative damage because in the brain the demand for molecular oxygen is significant, while the abundance of antioxidant defense enzymes is low. In AD, the brain undergoes increased oxidative stress from free radical damage, resulting in cellular dysfunction and neuronal degeneration, and subsequent symptoms of AD including dementia (Varadarajan et al., 2000). Sites where oxidative stress and neurodegeneration occur are associated with A-beta deposits (Butterfield, 1997; Markesbery, 1997). Free radical oxidative stress affecting neuronal lipids (phospholipid peroxidation) and causing protein oxidation is extensive, and may be correlated to A-beta deposits (Butterfield and Lauderback, 2002). The alteration of iron and copper homeostasis, and the resulting effect on oxidative stress, may also be involved in Alzheimer's disease (Moreira et al., 2005).

Inflammation is another major pathology that characterizes AD. Stress factors stemming from an oxidative imbalance and leading to inflammation, are deemed as one of the earliest events in AD pathogenesis. However, the classic inflammatory response, namely immunoglobulin and leukocyte infiltration is absent. Congregated with senile plaques are astrocytes and microglia, both of which exhibit a reactive response and increased expression of inflammatory markers. Some of these markers include MHC-I and –II, IL-1β, and TNF-α. Receptors for several cytokines and inflammatory agents also experience an increase in expression (Halliday et al., 2000). Inflammatory microglia may be activated by fibrillar A-beta (1-42) through the affinity of A-beta (1-16) for the C1q protein which subsequently binds to the microglial C1q receptor (Eikelenboom and Veerhuis, 1996). Microglia typically react first in the brain to injury or infection, and subsequently secrete acute-phase proteins and cytokines that recruit astrocytes. Of these

released cytokines, IL-1β and TNF-a are both known to up-regulate sPLA2-IIA in astrocytes. Treatment of cultured human astrocytes with Aβ(1-42) and IL-1β exhibited the same up-regulation of sPLA2-IIA mRNA (Moses et al., 2006).

NADPH Oxidase in Astrocytes

The NADPH oxidase family of enzymes was first identified in neutrophils and macrophages where it was shown to produce large amounts of superoxide. More recently, NADPH oxidase has been discovered in many other cell types, including astrocytes (Abramov et al., 2005). Amyloid beta peptides have been shown to activate NADPH oxidase in astrocytes (Abramov et al., 2004), which is not surprising because many neurodegenerative diseases are linked to oxidative stress (Simonian and Coyle, 1996). According to Abramov (2005), changes in calcium levels in astrocytes, whether from physiological calcium signals or ionophores, appears to be the factor contributing to activation of NADPH oxidase in astrocytes.

Components of NADPH Oxidase

NADPH oxidase has both membrane and cytoplasmic components, and is activated when all necessary components assemble at the plasma membrane. The cytoplasmic components include p47phox, p67phox, p40phox, and Rac GTP. The membrane components include gp91phox and p22phox. gp91phox, in its classical isoform, is now known as nox2, and there are several other nox isoforms (nox1, nox4, and nox3; (Dworakowski et al., 2006).

Many factors can activate the cytoplasmic components of NADPH oxidase, initiating the activation of the NADPH oxidase complex. Many methods can be used to activate the NADPH oxidase complex, including PMA, Aβ, and disrupting calcium levels.

p47phox is known to be activated upon association with other cytoplasmic components and phosphorylation by PKC-ζ (Dworakowski et al., 2006), with PI3-kinase is an essential element of the activation (Frey et al., 2006).

Activation of NADPH oxidase is known to result in p38 MAPK and ERK1/2 activation, as well as NF-kB activation. PKC itself activates ERK1/2 as well as PI3-kinase (Andersen et al., 2003). p38 MAPK is known to be sensitive to oxidative stress, and ERK1/2 has been shown to follow suit during NADPH oxidase stimulation and inhibition.

Pathways for NADPH Oxidase Activation

sPLA2 and cPLA2 are both up-regulated in oxidative stress and inflammatory processes, along with COX-2 and iNOS (Farooqui et al., 1999; Murakami et al., 1999). Eicosanoids and ROS are generated in pathological conditions like ischemia and Alzheimer's disease due to enhancement of AA metabolism. In the CNS, NADPH oxidase is a major source of ROS, and may be involved in the activation of sPLA2 gene expression (Sun et al., 2007; Jensen et al., 2009).

PKC and elevations in intracellular calcium levels also activate NOX (Abramov et al., 2005). IL-1β is known to activate p38 MAPK in human neutrophils (Suzuki et al., 2001), as well as in astrocytes (Wu et al., 2004). p38 MAPK, as well as ERK1/2, can

phosphorylate cytoplasmic components, initiating the translocation of p47phox and p67phox to the cell membrane and the activation of NOX (El Benna et al., 1996).

NF-kB activation by IL-1β has been demonstrated previously, and p38 MAPK activation occurs after IL-1β treatment (Vervoordeldonk et al., 1996). p38 MAPK is also involved in the activation of NOX, as well as other kinases including phosphatidyl inositol 3-kinase (PI3-K), ERK1/2, and PKC (Peng et al., 2005; Frey et al., 2006). PI3-kinases (PI3Ks) catalyze the conversion of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PI3K-δ has been shown to activate PKC-δ in endothelial cells, mediated by PIP3 production by cytokines, and resulting in NF-kB activation (Frey et al., 2006). PI3-kinase and Akt have also been shown to activate NOX through Akt phosphorylation of p47phox in neutrophils (Hoyal et al., 2003). ROS generated from NOX is known to participate in signaling in microglia and astrocytes (Pawate et al., 2004), and reactive oxygen intermediates can activate NF-kB (Schreck et al., 1991).

ERK1/2 is known to phosphorylate p47phox (El Benna et al., 1996). PI-3 Kinase has been shown in neutrophils to phosphorylate p47phox and lead to activation of NOX (Hoyal et al., 2003). PI-3 Kinase is also known for having a crucial role in cytokine induced activation of NF-kB through NOX in endothelial cells (Frey et al., 2006).

Botanical antioxidants: Apocynin, Resveratrol, and EGCG

Oxidative stress and inflammation both play crucial roles in a number of neurodegenerative disorders, including Alzheimer's disease. Various compounds have been identified with inherent anti-oxidative and anti-inflammatory effects, including

phenolic compounds from various botanical sources. These compounds may inhibit processes leading to ROS generation, or counteract the effects of ROS (Sun et al., 2008a). Three of these compounds used in our studies include apocynin, resveratrol, and epigallocatechin gallate (EGCG).

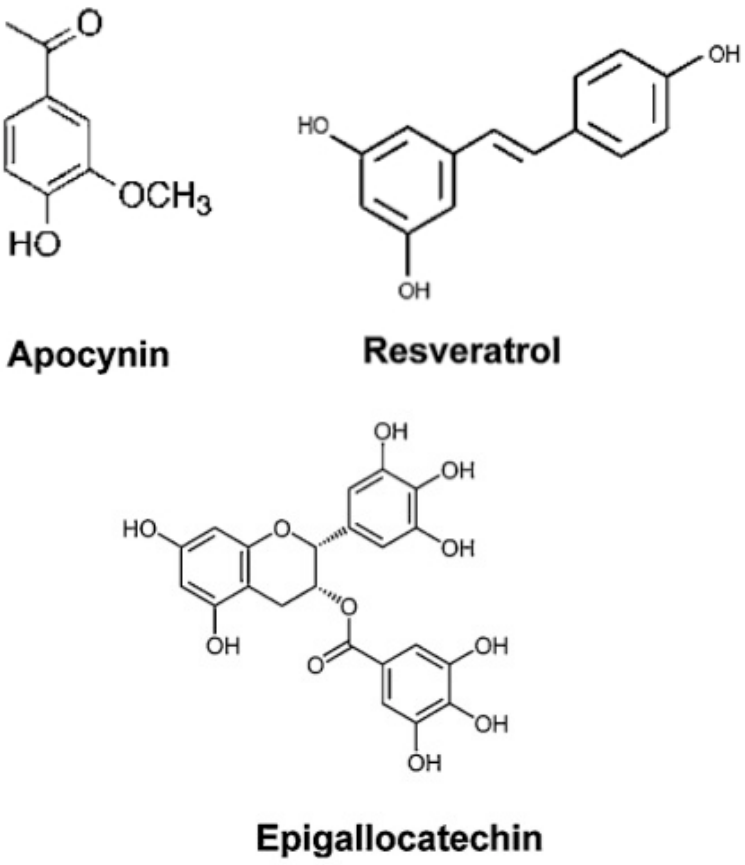

Figure 1.2 – Molecular structures of resveratrol, apocynin, and epigallocatechin-gallate

Apocynin

Apocynin (4-hydroxy-3-methoxyacetophenone) is an acetophenone (also called acetovanillone), isolated from the roots of *Apocynum cannabinum* (Canadian hemp), was first described by Schmiedeberg in 1883, and was used as a remedy for dropsy and heart

troubles (Stefanska and Pawliczak, 2008). In 1971, apocynin was identified in a discovery study for immunomodulatory constituents, derived from the *Picrorhiza kurroa* plant, which grows in the Himalayan mountains (Luchtefeld et al., 2008; Stefanska and Pawliczak, 2008). In the presence of myoloperoxidase, apocynin is thought to form a dimer as diapocynin, which is considered to be its active form intracellularly. However, this assumption has been disputed and there is no proof of diapocynin in the plasma after injection of apocynin (Wang et al., 2008).

Apocynin (as diapocynin) is a potent NOX inhibitor and has been shown to inhibit the intracellular assembly of NADPH oxidase in neutrophils and eosinophils (Stolk et al., 1994). COX-2 synthesis and activity are attenuated by apocynin; part of this is due to its ability to decrease the intracellular reduced/oxidized glutathione ratio (GSH/GSSG), as well as preventing NF-κB activation (Barbieri et al., 2004). In animal models, apocynin has been shown to limit inflammation in rheumatoid arthritis (Lafeber et al., 1999; Van den Worm et al., 2001) and to protect against oxidative damage due to global cerebral ischemia-reperfusion during both the early phase I/R and subsequent I/R (Wang et al., 2006; Suh et al., 2007). In a mouse model of ischemic stroke, certain doses (2.5 mg/kg) of apocynin given before reperfusion improved neurological function, reduced infarct volume, reduced the incidence of cerebral hemorrhage, and reduced mortality (Stefanska and Pawliczak, 2008).

Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a polyphenol known for its presence in red wines and its multitude of effects as an anti-oxidant, free radical scavenger, anti-

proliferator, differentiating factor, and anticancer, anti-inflammatory, angiogenetic, pro-apoptotic, and cardioprotective agent (Hung et al., 2002; Cucciolla et al., 2007)Sun et al., 2008). Resveratrol is known to affect cell membrane proteins and receptors including cAMP, growth factor protein tyrosine kinases, COX-2 and lipooxygenase, cell-surface adhesion molecules, androgen and estrogen receptors, and aryl hydrocarbon receptors. This polyphenol is known to affect transduction pathways including NF-kB, MAPK, PKCs, nitric oxide and nitric oxide synthase. Resveratrol is known to affect transcription and gene expression relating to activator protein-1, early growth response protein 1, the Rb-E2F/DP pathway, and the down-regulation of COX-2, 5-LOX, iNOS, ICAM-1, TNF, IL-1β, IL-6, IL-8, along with several genes involved in cell growth control (Cucciolla et al., 2007).

Resveratrol has been shown to reduce A-beta-induced toxicity in a dose-dependent manner (Han et al., 2004). Resveratrol is known to protect against global cerebral ischemic injury in gerbils (Wang et al., 2002) and against neurotoxicity induced by kainic acid (Wang et al., 2004). Resveratrol has also been shown to suppress NOX activity by reducing the membrane association of gp91phox and Rac1 (Chow et al., 2007).

Epigallocatechin gallate

Epigallocatechin gallate (EGCG) is a catechin well known as an important ingredient and major constituent of green tea. EGCG has been shown to act as a radical scavenger *in vitro*, as well as activating transcription factors to induce antioxidant effects through various mediators. EGCG may also be involved in the control of calcium

homeostasis (Ishige et al., 2001), MAPK and PKC activation (Chung et al., 2003; Levites et al., 2003), and antioxidant enzyme activation (Chen et al., 2000). EGCG is known for improving age-related cognitive decline, suppressing the generation of reactive oxygen species, and its neuroprotective capacity in models of Parkinson's Disease, cerebral ischemia/reperfusion injuries, and Alzheimer's disease (Mandel et al., 2004). Green tea extracts have shown to have neuroprotective effects against A-beta toxicity, and EGCG treatments alone had the same neuroprotective effect along with an inhibition of A-beta aggregation (Bastianetto et al., 2006). EGCG has been shown to promote the non-amyloidogenic alpha-secretase pathway of APP in neuronal cell cultures (Levites et al., 2002). EGCG has also been found to inhibit the translocation of cytosolic components of NOX (Nishikawa et al., 2007).

Bioinformatics

The third aspect of my doctoral studies included a fellowship in Bioinformatics with the National Library of Medicine (NLM). Although this fellowship did not lead directly to published data, the fellowship led to the development and delivery of training at the Pacific Symposium on Biocomputing in 2004 and 2005, as well as a prototype service during a two-month internship at the National Library of Medicine headquarters in Bethesda, Maryland. The focus of the NLM fellowship was Web Services as a solution to the problem of data integration between tools and researchers.

The core of the problem lies in (1) inconsistent interfaces and communication protocols, (2) a lack of structured data formats for both input and output of services, and (3) no central listing of services enabling service discovery and dynamic workflows.

With better methods of integrating scientific data services, turning data into results-oriented information can become a task that even non-technical researchers could perform. Web services is a solution that has garnered worldwide support in the science community as a means to overcoming these issues.

Web services make any tool or service available for programmatic access, making it possible for services to be integrated with each other and for the design of service workflows. The Semantic Web and the use of ontologies make services able to be found by other services and programs based on input, output, and function through service registries. Ontologies are an industry or field-based vocabulary with a way to define relationships between objects within the vocabulary, making it possible for not only humans to use common language when referring to data, but also machines. Ontologies are being developed in neuroscience and other fields, improving opportunities for more automated data and service integration. These technologies and developments allow researchers to discover new services based on data needing processed, and for applications to be able to automate the creation of workflows. Two projects fulfill this service architecture design, myGrid and BioMOBY, and several interfaces are available for accessing services and workflows through this software.

Web services use standard Internet protocols for interoperability with other resources, and offer the necessary architecture for flexible and expandable integration of diverse scientific tools. A web service consists of a server and client, where the server hosts the tool, service, or database, the client queries the server with request parameters (input), and the server sends the client a response (output). Typically web services make use of XML protocols such as SOAP, WSDL and either UDDI or RDF, allowing

communication between a service and any client implementing these protocols, including other services acting as clients to independent services. Web services can function without a registry, but the registry allows for services to be found either by a user manually looking for a particular service, or dynamically such as through workflow software.

New research methods generating large amounts of data require automated, high-throughput tools to process and glean insights from raw data. In neuroscience, methods involving DNA microarrays, proteomic studies, brain imaging data, and brain electrical recordings all generate large data sets that traditional labs aren't always prepared to handle alone. Web services are designed for scalability in processing large data sets because of the automated nature of their design. Using workflows to integrate with multiple web services also gives consistency in data processing over manually formatting data for multiple services. With the client-server architecture, web services can also make use of grid computing for computationally intensive calculations, spreading out processing across dispersed computers in a grid network.

Summary

Secretory phospholipase A2-IIA is well known for its role in many inflammatory diseases, and has also been implicated in several neurodegenerative diseases. Inflammatory factors including the cytokines IL-1β and TNF-α, known to be up-regulated in Alzheimer's disease, are factors that also induce sPLA2-IIA in cultured astrocytes. Reactive astrocytes, along with activated microglial cells and dystrophic neurites, frequently surround amyloid plaques, which is one of the landmarks of

Alzheimer's disease. Despite the implications of sPLA2-IIA in neurodegenerative diseases, no previous studies have shown evidence of sPLA2-IIA expression in AD brains. The first study, performed in this research project, characterized sPLA2-IIA expression in AD and ND brains (Chapter II). We started by partnering with the Sun Health Research Institute with access to postmortem human AD brains, allowing us to do both mRNA expression and immunohistochemistry studies to determine the sPLA2-IIA expression profile, expression levels, and localization.

Additionally, the relationship between sPLA2-IIA expression in astrocytes and NADPH oxidase activation is not well understood. In the second study (Chapter III), we used IL-1β to induce sPLA2-IIA expression in cultured rat astrocytes as a model for the up-regulation of sPLA2-IIA. We identified signaling molecules, i.e., protein kinases, involved in cytokine-induced sPLA2-IIA induction. We tested whether botanical antioxidants are able to down-regulate sPLA2-IIA expression and whether their mechanism of action involves the NADPH oxidase pathway.

These studies together provide novel details into sPLA2-IIA and astrocytes, specifically as it relates to Alzheimer's disease. In chapter 2, our findings were the first to identify sPLA2-IIA up-regulation in Alzheimer's and specifically what parts of the brain sPLA2-IIA is more highly expressed. The results in chapter 2 from cytokine and A-β activation of sPLA2-IIA expression in cultured human astrocytes prepared a foundation for work done in chapter 3, where we utilized IL-1β as a stimulating cytokine for sPLA2-IIA expression. Chapter 3 also suggests NADPH oxidase related pathways involved in the IL-1β stimulated expression of sPLA2-IIA.

References

Abramov AY, Canevari L, Duchen MR (2004) Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 24:565-575.

Abramov AY, Jacobson J, Wientjes F, Hothersall J, Canevari L, Duchen MR (2005) Expression and modulation of an NADPH oxidase in mammalian astrocytes. J Neurosci 25:9176-9184.

Adibhatla RM, Hatcher JF (2007) Secretory phospholipase A2 IIA is up-regulated by TNF-alpha and IL-1alpha/beta after transient focal cerebral ischemia in rat. Brain research 1134:199-205.

Akiba S, Hatazawa R, Ono K, Kitatani K, Hayama M, Sato T (2001) Secretory phospholipase A2 mediates cooperative prostaglandin generation by growth factor and cytokine independently of preceding cytosolic phospholipase A2 expression in rat gastric epithelial cells. The Journal of biological chemistry 276:21854-21862.

Andersen JM, Myhre O, Fonnum F (2003) Discussion of the role of the extracellular signal-regulated kinase-phospholipase A2 pathway in production of reactive oxygen species in Alzheimer's disease. Neurochemical research 28:319-326.

Andreani M, Olivier JL, Berenbaum F, Raymondjean M, Bereziat G (2000) Transcriptional regulation of inflammatory secreted phospholipases A(2). Biochimica et biophysica acta 1488:149-158.

Balboa MA, Balsinde J, Winstead MV, Tischfield JA, Dennis EA (1996) Novel group V phospholipase A2 involved in arachidonic acid mobilization in murine P388D1 macrophages. The Journal of biological chemistry 271:32381-32384.

Balestrieri B, Hsu VW, Gilbert H, Leslie CC, Han WK, Bonventre JV, Arm JP (2006) Group V secretory phospholipase A2 translocates to the phagosome after zymosan stimulation of mouse peritoneal macrophages and regulates phagocytosis. The Journal of biological chemistry 281:6691-6698.

Barbieri SS, Cavalca V, Eligini S, Brambilla M, Caiani A, Tremoli E, Colli S (2004) Apocynin prevents cyclooxygenase 2 expression in human monocytes through NADPH oxidase and glutathione redox-dependent mechanisms. Free radical biology & medicine 37:156-165.

Bastianetto S, Yao ZX, Papadopoulos V, Quirion R (2006) Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. The European journal of neuroscience 23:55-64.

Bezzine S, Koduri RS, Valentin E, Murakami M, Kudo I, Ghomashchi F, Sadilek M, Lambeau G, Gelb MH (2000) Exogenously added human group X secreted phospholipase A(2) but not the group IB, IIA, and V enzymes efficiently release arachidonic acid from adherent mammalian cells. The Journal of biological chemistry 275:3179-3191.

Bingham CO, 3rd, Fijneman RJ, Friend DS, Goddeau RP, Rogers RA, Austen KF, Arm JP (1999) Low molecular weight group IIA and group V phospholipase A(2) enzymes have different intracellular locations in mouse bone marrow-derived mast cells. The Journal of biological chemistry 274:31476-31484.

Boilard E, Bourgoin SG, Bernatchez C, Poubelle PE, Surette ME (2003) Interaction of low molecular weight group IIA phospholipase A2 with apoptotic human T cells: role of heparan sulfate proteoglycans. Faseb J 17:1068-1080.

Bostrom MA, Boyanovsky BB, Jordan CT, Wadsworth MP, Taatjes DJ, de Beer FC, Webb NR (2007) Group v secretory phospholipase A2 promotes atherosclerosis: evidence from genetically altered mice. Arteriosclerosis, thrombosis, and vascular biology 27:600-606.

Burke JE, Dennis EA (2009a) Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res 50 Suppl:S237-242.

Burke JE, Dennis EA (2009b) Phospholipase A2 biochemistry. Cardiovasc Drugs Ther 23:49-59.

Butterfield DA (1997) beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. Chem Res Toxicol 10:495-506.

Butterfield DA, Lauderback CM (2002) Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radic Biol Med 32:1050-1060.

Carey MC, Small DM, Bliss CM (1983) Lipid digestion and absorption. Annu Rev Physiol 45:651-677.

Chen C, Yu R, Owuor ED, Kong AN (2000) Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 23:605-612.

Chen J, Engle SJ, Seilhamer JJ, Tischfield JA (1994a) Cloning and recombinant expression of a novel human low molecular weight Ca(2+)-dependent phospholipase A2. The Journal of biological chemistry 269:2365-2368.

Chen J, Engle SJ, Seilhamer JJ, Tischfield JA (1994b) Cloning, expression and partial characterization of a novel rat phospholipase A2. Biochimica et biophysica acta 1215:115-120.

Chen J, Shao C, Lazar V, Srivastava CH, Lee WH, Tischfield JA (1997) Localization of group IIc low molecular weight phospholipase A2 mRNA to meiotic cells in the mouse. J Cell Biochem 64:369-375.

Chow SE, Hshu YC, Wang JS, Chen JK (2007) Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. J Appl Physiol 102:1520-1527.

Chung JH, Han JH, Hwang EJ, Seo JY, Cho KH, Kim KH, Youn JI, Eun HC (2003) Dual mechanisms of green tea extract (EGCG)-induced cell survival in human epidermal keratinocytes. Faseb J 17:1913-1915.

Cucciolla V, Borriello A, Oliva A, Galletti P, Zappia V, Della Ragione F (2007) Resveratrol: from basic science to the clinic. Cell cycle (Georgetown, Tex 6:2495-2510.

Cupillard L, Koumanov K, Mattei MG, Lazdunski M, Lambeau G (1997) Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A2. The Journal of biological chemistry 272:15745-15752.

Dennis EA (1994) Diversity of group types, regulation, and function of phospholipase A2. The Journal of biological chemistry 269:13057-13060.

Dworakowski R, Anilkumar N, Zhang M, Shah AM (2006) Redox signalling involving NADPH oxidase-derived reactive oxygen species. Biochemical Society transactions 34:960-964.

Eikelenboom P, Veerhuis R (1996) The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. Neurobiol Aging 17:673-680.

El Benna J, Han J, Park JW, Schmid E, Ulevitch RJ, Babior BM (1996) Activation of p38 in stimulated human neutrophils: phosphorylation of the oxidase component p47phox by p38 and ERK but not by JNK. Archives of biochemistry and biophysics 334:395-400.

Farbman AI, Brann JH, Rozenblat A, Rochlin MW, Weiler E, Bhattacharyya M (2004) Developmental expression of neurotrophin receptor genes in rat geniculate ganglion neurons. J Neurocytol 33:331-343.

Farooqui AA, Horrocks LA (2006) Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12:245-260.

Farooqui AA, Yang HC, Horrocks L (1997) Involvement of phospholipase A2 in neurodegeneration. Neurochem Int 30:517-522.

Farooqui AA, Litsky ML, Farooqui T, Horrocks LA (1999) Inhibitors of intracellular phospholipase A2 activity: their neurochemical effects and therapeutical importance for neurological disorders. Brain Res Bull 49:139-153.

Floyd RA (1999) Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 222:236-245.

Floyd RA, Carney JM (1992) Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann Neurol 32 Suppl:S22-27.

Frey RS, Gao X, Javaid K, Siddiqui SS, Rahman A, Malik AB (2006) Phosphatidylinositol 3-kinase gamma signaling through protein kinase Czeta induces NADPH oxidase-mediated oxidant generation and NF-kappaB activation in endothelial cells. The Journal of biological chemistry 281:16128-16138.

Fuentes L, Hernandez M, Fernandez-Aviles FJ, Crespo MS, Nieto ML (2002) Cooperation between secretory phospholipase A2 and TNF-receptor superfamily signaling: implications for the inflammatory response in atherogenesis. Circ Res 91:681-688.

Fujioka D, Saito Y, Kobayashi T, Yano T, Tezuka H, Ishimoto Y, Suzuki N, Yokota Y, Nakamura T, Obata JE, Kanazawa M, Kawabata K, Hanasaki K, Kugiyama K (2008) Reduction in myocardial ischemia/reperfusion injury in group X secretory phospholipase A2-deficient mice. Circulation 117:2977-2985.

Gella A, Durany N (2009) Oxidative stress in Alzheimer disease. Cell Adh Migr 3.

Ghosh M, Tucker DE, Burchett SA, Leslie CC (2006) Properties of the Group IV phospholipase A2 family. Prog Lipid Res 45:487-510.

Halliday G, Robinson SR, Shepherd C, Kril J (2000) Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. Clinical and experimental pharmacology & physiology 27:1-8.

Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV (2003) Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. The Journal of biological chemistry 278:24153-24163.

Han YS, Zheng WH, Bastianetto S, Chabot JG, Quirion R (2004) Neuroprotective effects of resveratrol against beta-amyloid-induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. British journal of pharmacology 141:997-1005.

Hanasaki K, Arita H (2002) Phospholipase A2 receptor: a regulator of biological functions of secretory phospholipase A2. Prostaglandins Other Lipid Mediat 68-69:71-82.

Hanasaki K, Ono T, Saiga A, Morioka Y, Ikeda M, Kawamoto K, Higashino K, Nakano K, Yamada K, Ishizaki J, Arita H (1999) Purified group X secretory phospholipase A(2) induced prominent release of arachidonic acid from human myeloid leukemia cells. The Journal of biological chemistry 274:34203-34211.

Henderson WR, Jr., Chi EY, Bollinger JG, Tien YT, Ye X, Castelli L, Rubtsov YP, Singer AG, Chiang GK, Nevalainen T, Rudensky AY, Gelb MH (2007) Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. The Journal of experimental medicine 204:865-877.

Hirabayashi T, Murayama T, Shimizu T (2004) Regulatory mechanism and physiological role of cytosolic phospholipase A2. Biol Pharm Bull 27:1168-1173.

Ho IC, Arm JP, Bingham CO, 3rd, Choi A, Austen KF, Glimcher LH (2001) A novel group of phospholipase A2s preferentially expressed in type 2 helper T cells. The Journal of biological chemistry 276:18321-18326.

Hoyal CR, Gutierrez A, Young BM, Catz SD, Lin JH, Tsichlis PN, Babior BM (2003) Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. Proceedings of the National Academy of Sciences of the United States of America 100:5130-5135.

Hung LM, Su MJ, Chu WK, Chiao CW, Chan WF, Chen JK (2002) The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. British journal of pharmacology 135:1627-1633.

Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P (2001) Phospholipase A(2) in vascular disease. Circulation research 89:298-304.

Ikeno Y, Konno N, Cheon SH, Bolchi A, Ottonello S, Kitamoto K, Arioka M (2005) Secretory phospholipases A2 induce neurite outgrowth in PC12 cells through lysophosphatidylcholine generation and activation of G2A receptor. The Journal of biological chemistry 280:28044-28052.

Ishige K, Schubert D, Sagara Y (2001) Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free radical biology & medicine 30:433-446.

Ishizaki J, Suzuki N, Higashino K, Yokota Y, Ono T, Kawamoto K, Fujii N, Arita H, Hanasaki K (1999) Cloning and characterization of novel mouse and human secretory phospholipase A(2)s. The Journal of biological chemistry 274:24973-24979.

Jamal OS, Conaghan PG, Cunningham AM, Brooks PM, Munro VF, Scott KF (1998) Increased expression of human type IIa secretory phospholipase A2 antigen in arthritic synovium. Ann Rheum Dis 57:550-558.

Jensen MD, Sheng W, Simonyi A, Johnson GS, Sun AY, Sun GY (2009) Involvement of oxidative pathways in cytokine-induced secretory phospholipase A2-IIA in astrocytes. Neurochem Int.

Jiang J, Neubauer BL, Graff JR, Chedid M, Thomas JE, Roehm NW, Zhang S, Eckert GJ, Koch MO, Eble JN, Cheng L (2002) Expression of group IIA secretory phospholipase A2 is elevated in prostatic intraepithelial neoplasia and adenocarcinoma. The American journal of pathology 160:667-671.

Kashiwagi M, Friess H, Uhl W, Berberat P, Abou-Shady M, Martignoni M, Anghelacopoulos SE, Zimmermann A, Buchler MW (1999) Group II and IV phospholipase A(2) are produced in human pancreatic cancer cells and influence prognosis. Gut 45:605-612.

Kolko M, Christoffersen NR, Varoqui H, Bazan NG (2005) Expression and induction of secretory phospholipase A2 group IB in brain. Cellular and molecular neurobiology 25:1107-1122.

Kumar A, Sekharudu C, Ramakrishnan B, Dupureur CM, Zhu H, Tsai MD, Sundaralingam M (1994) Structure and function of the catalytic site mutant Asp 99 Asn of phospholipase A2: absence of the conserved structural water. Protein Sci 3:2082-2088.

Lafeber FP, Beukelman CJ, van den Worm E, van Roy JL, Vianen ME, van Roon JA, van Dijk H, Bijlsma JW (1999) Apocynin, a plant-derived, cartilage-saving drug, might be useful in the treatment of rheumatoid arthritis. Rheumatology (Oxford) 38:1088-1093.

Lambeau G, Lazdunski M (1999) Receptors for a growing family of secreted phospholipases A2. Trends in pharmacological sciences 20:162-170.

Lambeau G, Gelb MH (2008) Biochemistry and physiology of mammalian secreted phospholipases A2. Annu Rev Biochem 77:495-520.

Levites Y, Amit T, Youdim MB, Mandel S (2002) Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (-)-

epigallocatechin 3-gallate neuroprotective action. The Journal of biological chemistry 277:30574-30580.

Levites Y, Amit T, Mandel S, Youdim MB (2003) Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (-)-epigallocatechin-3-gallate. Faseb J 17:952-954.

Lin MK, Farewell V, Vadas P, Bookman AA, Keystone EC, Pruzanski W (1996) Secretory phospholipase A2 as an index of disease activity in rheumatoid arthritis. Prospective double blind study of 212 patients. J Rheumatol 23:1162-1166.

Lin TN, Wang Q, Simonyi A, Chen JJ, Cheung WM, He YY, Xu J, Sun AY, Hsu CY, Sun GY (2004) Induction of secretory phospholipase A2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. J Neurochem 90:637-645.

Liu Z, Lu X, Wang H, You XJ, Gao QX, Cui YH (2007) Group II subfamily secretory phospholipase A2 enzymes: expression in chronic rhinosinusitis with and without nasal polyps. Allergy 62:999-1006.

Luchtefeld R, Luo R, Stine K, Alt ML, Chernovitz PA, Smith RE (2008) Dose formulation and analysis of diapocynin. J Agric Food Chem 56:301-306.

Mandel S, Weinreb O, Amit T, Youdim MB (2004) Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. Journal of neurochemistry 88:1555-1569.

Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free radical biology & medicine 23:134-147.

Masuda S, Yamamoto K, Hirabayashi T, Ishikawa Y, Ishii T, Kudo I, Murakami M (2008) Human group III secreted phospholipase A2 promotes neuronal outgrowth and survival. The Biochemical journal 409:429-438.

Masuda S, Murakami M, Takanezawa Y, Aoki J, Arai H, Ishikawa Y, Ishii T, Arioka M, Kudo I (2005) Neuronal expression and neuritogenic action of group X secreted phospholipase A2. The Journal of biological chemistry 280:23203-23214.

McGeer EG, Klegeris A, McGeer PL (2005) Inflammation, the complement system and the diseases of aging. Neurobiol Aging 26 Suppl 1:94-97.

Moreira PI, Siedlak SL, Aliev G, Zhu X, Cash AD, Smith MA, Perry G (2005) Oxidative stress mechanisms and potential therapeutics in Alzheimer disease. J Neural Transm 112:921-932.

Moses GS, Jensen MD, Lue LF, Walker DG, Sun AY, Simonyi A, Sun GY (2006) Secretory PLA2-IIA: a new inflammatory factor for Alzheimer's disease. J Neuroinflammation 3:28.

Mounier CM, Wendum D, Greenspan E, Flejou JF, Rosenberg DW, Lambeau G (2008) Distinct expression pattern of the full set of secreted phospholipases A2 in human colorectal adenocarcinomas: sPLA2-III as a biomarker candidate. Br J Cancer 98:587-595.

Mounier CM, Ghomashchi F, Lindsay MR, James S, Singer AG, Parton RG, Gelb MH (2004) Arachidonic acid release from mammalian cells transfected with human groups IIA and X secreted phospholipase A(2) occurs predominantly during the secretory process and with the involvement of cytosolic phospholipase A(2)-alpha. The Journal of biological chemistry 279:25024-25038.

Murakami M, Kudo I (2002) Phospholipase A2. J Biochem 131:285-292.

Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I (1997) Regulatory functions of phospholipase A2. Critical reviews in immunology 17:225-283.

Murakami M, Kambe T, Shimbara S, Yamamoto S, Kuwata H, Kudo I (1999) Functional association of type IIA secretory phospholipase A(2) with the glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan in the cyclooxygenase-2-mediated delayed prostanoid-biosynthetic pathway. J Biol Chem 274:29927-29936.

Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T, Kudo I (2005) Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2). The Journal of biological chemistry 280:24987-24998.

Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfield JA, Kudo I (1998) The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. The Journal of biological chemistry 273:14411-14423.

Murakami M, Yoshihara K, Shimbara S, Lambeau G, Singer A, Gelb MH, Sawada M, Inagaki N, Nagai H, Kudo I (2002a) Arachidonate release and eicosanoid generation by group IIE phospholipase A(2). Biochemical and biophysical research communications 292:689-696.

Murakami M, Yoshihara K, Shimbara S, Sawada M, Inagaki N, Nagai H, Naito M, Tsuruo T, Moon TC, Chang HW, Kudo I (2002b) Group IID heparin-binding secretory phospholipase A(2) is expressed in human colon carcinoma cells and

human mast cells and up-regulated in mouse inflammatory tissues. Eur J Biochem 269:2698-2707.

Murakami M, Masuda S, Shimbara S, Bezzine S, Lazdunski M, Lambeau G, Gelb MH, Matsukura S, Kokubu F, Adachi M, Kudo I (2003) Cellular arachidonate-releasing function of novel classes of secretory phospholipase A2s (groups III and XII). The Journal of biological chemistry 278:10657-10667.

Murakami M, Yoshihara K, Shimbara S, Lambeau G, Gelb MH, Singer AG, Sawada M, Inagaki N, Nagai H, Ishihara M, Ishikawa Y, Ishii T, Kudo I (2002c) Cellular arachidonate-releasing function and inflammation-associated expression of group IIF secretory phospholipase A2. The Journal of biological chemistry 277:19145-19155.

Nakashima S, Kitamoto K, Arioka M (2004) The catalytic activity, but not receptor binding, of sPLA2s plays a critical role for neurite outgrowth induction in PC12 cells. Brain research 1015:207-211.

Nguyen MD, Julien JP, Rivest S (2002) Innate immunity: the missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci 3:216-227.

Ni Z, Okeley NM, Smart BP, Gelb MH (2006) Intracellular actions of group IIA secreted phospholipase A2 and group IVA cytosolic phospholipase A2 contribute to arachidonic acid release and prostaglandin production in rat gastric mucosal cells and transfected human embryonic kidney cells. The Journal of biological chemistry 281:16245-16255.

Nishikawa H, Wakano K, Kitani S (2007) Inhibition of NADPH oxidase subunits translocation by tea catechin EGCG in mast cell. Biochemical and biophysical research communications 362:504-509.

Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, Smith MA (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J Neurosci 19:1959-1964.

Ohtsuki M, Taketomi Y, Arata S, Masuda S, Ishikawa Y, Ishii T, Takanezawa Y, Aoki J, Arai H, Yamamoto K, Kudo I, Murakami M (2006) Transgenic expression of group V, but not group X, secreted phospholipase A2 in mice leads to neonatal lethality because of lung dysfunction. The Journal of biological chemistry 281:36420-36433.

Oslund RC, Cermak N, Gelb MH (2008) Highly specific and broadly potent inhibitors of mammalian secreted phospholipases A2. J Med Chem 51:4708-4714.

Pawate S, Shen Q, Fan F, Bhat NR (2004) Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. Journal of neuroscience research 77:540-551.

Peng T, Lu X, Feng Q (2005) Pivotal role of gp91phox-containing NADH oxidase in lipopolysaccharide-induced tumor necrosis factor-alpha expression and myocardial depression. Circulation 111:1637-1644.

Polazzi E, Contestabile A (2002) Reciprocal interactions between microglia and neurons: from survival to neuropathology. Rev Neurosci 13:221-242.

Probst A, Langui D, Ulrich J (1991) Alzheimer's disease: a description of the structural lesions. Brain Pathol 1:229-239.

Reid RC (2005) Inhibitors of secretory phospholipase A2 group IIA. Curr Med Chem 12:3011-3026.

Reynolds A, Laurie C, Mosley RL, Gendelman HE (2007) Oxidative stress and the pathogenesis of neurodegenerative disorders. Int Rev Neurobiol 82:297-325.

Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, Arm JP (2004) Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. The Journal of biological chemistry 279:16488-16494.

Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. The EMBO journal 10:2247-2258.

Schwab C, McGeer PL (2008) Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. J Alzheimers Dis 13:359-369.

Scott DL, White SP, Otwinowski Z, Yuan W, Gelb MH, Sigler PB (1990) Interfacial catalysis: the mechanism of phospholipase A2. Science 250:1541-1546.

Selkoe DJ (1996) Amyloid beta-protein and the genetics of Alzheimer's disease. The Journal of biological chemistry 271:18295-18298.

Shaftel SS, Kyrkanides S, Olschowka JA, Miller JN, Johnson RE, O'Banion MK (2007) Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. The Journal of clinical investigation 117:1595-1604.

Shirai Y, Ito M (2004) Specific differential expression of phospholipase A2 subtypes in rat cerebellum. J Neurocytol 33:297-307.

Simonian NA, Coyle JT (1996) Oxidative stress in neurodegenerative diseases. Annual review of pharmacology and toxicology 36:83-106.

Stefanska J, Pawliczak R (2008) Apocynin: molecular aptitudes. Mediators Inflamm 2008:106507.

Stolk J, Hiltermann TJ, Dijkman JH, Verhoeven AJ (1994) Characteristics of the inhibition of NADPH oxidase activation in neutrophils by apocynin, a methoxy-substituted catechol. Am J Respir Cell Mol Biol 11:95-102.

Suh SW, Gum ET, Hamby AM, Chan PH, Swanson RA (2007) Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. The Journal of clinical investigation 117:910-918.

Sun AY, Wang Q, Simonyi A, Sun GY (2008) Botanical phenolics and brain health. Neuromolecular Med 10:259-274.

Sun GY, Horrocks LA, Farooqui AA (2007) The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. J Neurochem 103:1-16.

Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 45:205-213.

Suzuki K, Hino M, Kutsuna H, Hato F, Sakamoto C, Takahashi T, Tatsumi N, Kitagawa S (2001) Selective activation of p38 mitogen-activated protein kinase cascade in human neutrophils stimulated by IL-1beta. J Immunol 167:5940-5947.

Suzuki N, Ishizaki J, Yokota Y, Higashino K, Ono T, Ikeda M, Fujii N, Kawamoto K, Hanasaki K (2000) Structures, enzymatic properties, and expression of novel human and mouse secretory phospholipase A(2)s. The Journal of biological chemistry 275:5785-5793.

Tischfield JA (1997) A reassessment of the low molecular weight phospholipase A2 gene family in mammals. J Biol Chem 272:17247-17250.

Tischfield JA, Xia YR, Shih DM, Klisak I, Chen J, Engle SJ, Siakotos AN, Winstead MV, Seilhamer JJ, Allamand V, Gyapay G, Lusis AJ (1996) Low-molecular-weight, calcium-dependent phospholipase A2 genes are linked and map to homologous chromosome regions in mouse and human. Genomics 32:328-333.

Vadas P, Browning J, Edelson J, Pruzanski W (1993) Extracellular phospholipase A2 expression and inflammation: the relationship with associated disease states. J Lipid Mediat 8:1-30.

Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G (1999) On the diversity of secreted phospholipases A(2). Cloning, tissue distribution, and functional expression of two novel mouse group II enzymes. The Journal of biological chemistry 274:31195-31202.

Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G (2000) Novel human secreted phospholipase A(2) with homology to the group III bee venom enzyme. The Journal of biological chemistry 275:7492-7496.

Van den Worm E, Beukelman CJ, Van den Berg AJ, Kroes BH, Labadie RP, Van Dijk H (2001) Effects of methoxylation of apocynin and analogs on the inhibition of reactive oxygen species production by stimulated human neutrophils. Eur J Pharmacol 433:225-230.

Varadarajan S, Yatin S, Aksenova M, Butterfield DA (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. J Struct Biol 130:184-208.

Verheij HM, Slotboom AJ, de Haas GH (1981) Structure and function of phospholipase A2. Rev Physiol Biochem Pharmacol 91:91-203.

Vervoordeldonk MJ, Pineda Torra IM, Aarsman AJ, van den Bosch H (1996) Aspirin inhibits expression of the interleukin-1beta-inducible group II phospholipase A2. FEBS letters 397:108-112.

Wang Q, Yu S, Simonyi A, Rottinghaus G, Sun GY, Sun AY (2004) Resveratrol protects against neurotoxicity induced by kainic acid. Neurochemical research 29:2105-2112.

Wang Q, Tompkins KD, Simonyi A, Korthuis RJ, Sun AY, Sun GY (2006) Apocynin protects against global cerebral ischemia-reperfusion-induced oxidative stress and injury in the gerbil hippocampus. Brain research 1090:182-189.

Wang Q, Xu J, Rottinghaus GE, Simonyi A, Lubahn D, Sun GY, Sun AY (2002) Resveratrol protects against global cerebral ischemic injury in gerbils. Brain research 958:439-447.

Wang Q, Smith RE, Luchtefeld R, Sun AY, Simonyi A, Luo R, Sun GY (2008) Bioavailability of apocynin through its conversion to glycoconjugate but not to diapocynin. Phytomedicine 15:496-503.

Wei S, Ong WY, Thwin MM, Fong CW, Farooqui AA, Gopalakrishnakone P, Hong W (2003) Group IIA secretory phospholipase A2 stimulates exocytosis and neurotransmitter release in pheochromocytoma-12 cells and cultured rat hippocampal neurons. Neuroscience 121:891-898.

Wijewickrama GT, Kim JH, Kim YJ, Abraham A, Oh Y, Ananthanarayanan B, Kwatia M, Ackerman SJ, Cho W (2006a) Systematic evaluation of transcellular activities of secretory phospholipases A2. High activity of group V phospholipases A2 to induce eicosanoid biosynthesis in neighboring inflammatory cells. The Journal of biological chemistry 281:10935-10944.

Wijewickrama GT, Albanese A, Kim YJ, Oh YS, Murray PS, Takayanagi R, Tobe T, Masuda S, Murakami M, Kudo I, Ucker DS, Murray D, Cho W (2006b) Unique membrane interaction mode of group IIF phospholipase A2. The Journal of biological chemistry 281:32741-32754.

Wu CY, Hsieh HL, Jou MJ, Yang CM (2004) Involvement of p42/p44 MAPK, p38 MAPK, JNK and nuclear factor-kappa B in interleukin-1beta-induced matrix metalloproteinase-9 expression in rat brain astrocytes. Journal of neurochemistry 90:1477-1488.

Xu J, Weng YI, Simonyi A, Krugh BW, Liao Z, Weisman GA, Sun GY (2002) Role of PKC and MAPK in cytosolic PLA2 phosphorylation and arachadonic acid release in primary murine astrocytes. J Neurochem 83:259-270.

Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Kishino J, Sakaguchi G, Itoh N, Hori Y (2002a) Group IB secretory phospholipase A(2)induces cell death in the cultured cortical neurons: a possible involvement of its binding sites. Brain research 949:197-201.

Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Takasu N, Tanaka K, Gemba T, Hori Y (2002b) Human group IIA secretory phospholipase A2 induces neuronal cell death via apoptosis. Mol Pharmacol 61:114-126.

Zaleska MM, Floyd RA (1985) Regional lipid peroxidation in rat brain in vitro: possible role of endogenous iron. Neurochemical research 10:397-410.

Chapter 2

SECRETORY PLA_2-IIA: A NEW INFLAMMATORY FACTOR FOR ALZHEIMER'S DISEASE*

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Abstract

Secretory phospholipase A_2-IIA ($sPLA_2$-IIA) is an inflammatory protein known to play a role in the pathogenesis of many inflammatory diseases. Although this enzyme has also been implicated in the pathogenesis of neurodegenerative diseases, there has not been a direct demonstration of its expression in diseased human brain. In this study, we show that $sPLA_2$-IIA mRNA is up-regulated in Alzheimer's disease (AD) brains as compared to non-demented elderly brains (ND). We also report a higher percentage of $sPLA_2$-IIA-immunoreactive astrocytes present in AD hippocampus and inferior temporal gyrus (ITG). In ITG, the majority of $sPLA_2$-IIA-positive astrocytes were associated with amyloid b (Ab)-containing plaques. Studies with human astrocytes in culture demonstrated the ability of oligomeric $A\beta_{1-42}$ and interleukin-1b (IL-1β) to induce $sPLA_2$-IIA mRNA expression, indicating that this gene is among those induced by inflammatory cytokines. Since exogenous $sPLA_2$-IIA has been shown to cause neuronal injury, understanding the mechanism(s) and physiological consequences of $sPLA_2$-IIA upregulation in AD brain may facilitate the development of novel therapeutic strategies to inhibit the inflammatory responses and to retard the progression of the disease.

Background

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease affecting the aging population, and is characterized by memory loss and decline in cognitive functions. Some of the characteristic landmarks of the disease include neurofibrillary tangles (Silvestrelli et al., 2006) and amyloid plaques, which are frequently surrounded by reactive astrocytes and activated microglial cells as well as dystrophic neurites (Pike et al., 1995; Jefferies et al., 1996). The presence of activated glial cells and the increase in inflammation-associated proteins in AD brain support the neuroinflammatory nature of this disease (Hu et al., 1998; McGeer and McGeer, 2003; Streit, 2004; Mrak and Griffin, 2005; Walker and Lue, 2005; Griffin, 2006). Increased amounts or deposits of inflammatory proteins such as the classical and alternative complement proteins and acute phase reactant proteins have been reported in AD brains, as have increased microglial expression of the major histocompatibility complex (MHC) antigens (Akiyama et al., 2000). Although the underlying mechanism(s) for neuroinflammation in AD brain is not clearly understood, there is considerable evidence supporting a role for specific forms of amyloid beta peptide (Aβ) in inducing production of pro-inflammatory cytokines by microglia and astrocytes (Akama et al., 1998; Hu et al., 1998; Cacquevel et al., 2004; Walker et al., 2006). Therefore, understanding the mechanisms that modulate neuroinflammatory responses and their impact on neuronal degenerative processes may help to uncover important elements of the disease and to develop new treatment strategies (Stuchbury and Munch, 2005; Craft et al., 2006; Ranaivo et al., 2006).

The phospholipases A_2 (PLA_2) belong to a family of enzymes that are widely expressed in many types of mammalian cells (Murakami and Kudo, 2002b). These enzymes not only play a role in maintenance of cell membrane phospholipids, but are also actively involved in the production of arachidonic acid (AA), the precursor for prostanoids (Bazan, 2003, 2005). Among more than 20 different forms of PLA_2 identified, there is considerable attention on the group IV calcium-dependent cytosolic PLA_2 ($cPLA_2$) and the group II secretory PLA_2 ($sPLA_2$). Both groups of PLA_2 can participate in the oxidative and inflammatory responses in neurodegenerative diseases (Farooqui et al., 1997b; Touqui and Alaoui-El-Azher, 2001; Balboa et al., 2002; Hashmi et al., 2004; Sun et al., 2004; Sun et al., 2005; Adibhatla and Hatcher, 2006). Although previous studies have demonstrated an increase in mRNA expression (Colangelo et al., 2002) and immunoreactivity of $cPLA_2$ in AD brains (Stephenson et al., 1996; Stephenson et al., 1999; Colangelo et al., 2002), studies to relate $sPLA_2$-IIA expression with AD have been lacking. In the periphery, $sPLA_2$-IIA is regarded as an inflammatory protein, and is involved in inflammatory diseases such as arthritis, atherosclerosis, acute lung injury, sepsis and cancer (Valentin and Lambeau, 2000; Hurt-Camejo et al., 2001; Touqui and Alaoui-El-Azher, 2001; Taketo and Sonoshita, 2002; Niessen et al., 2003). Secretory $sPLA_2$-IIA cannot be studied in transgenic mouse models of AD due to a frameshift mutation of this gene in many mouse strains (Kennedy et al., 1995). However, studies with rat models of brain injury have demonstrated an increase in $sPLA_2$-IIA expression associated with different forms of neuronal insults, including cerebral ischemia (Yagami et al., 2002a; Lin et al., 2004) as well as other types of neuronal injuries (Thwin et al., 2003; Cunningham et al., 2004).

In this report, we provide data demonstrating up-regulation of $sPLA_2$-IIA mRNA and protein expression in reactive astrocytes in AD brains as compared to age-matched non-demented (ND) control brains. In addition, studies with human astrocytes demonstrated the induction of $sPLA_2$-IIA mRNA by pro-inflammatory cytokines and Ab, further supporting an inflammatory role of this enzyme in AD brain.

Methods

Human brain tissue

Paraformaldehyde-fixed brain sections for immunohistochemistry were obtained from the Brain Bank of the Sun Health Research Institute (Sun City, AZ). Patients were classified as AD or ND cases by the neuropathological criteria of the Consortium to Establish a Registry for AD (CERAD) and NIA-Reagan guidelines. Postmortem brain samples were obtained from 7 male and 9 female ND subjects and 5 male and 11 female AD subjects (Table 1). The mean age (years) for the AD cases was 86.25$\pm$ 8.22 and for the ND cases was 84.44 $\pm$ 6.74 (mean $\pm$ SD), and the mean postmortem interval (hours) for AD cases was 2.59 $\pm$ 0.45 and for ND cases was 2.63 $\pm$ 0.62 (mean $\pm$ SD).

Stimulation of sPLA2-IIA mRNA expression in astrocytes from human post-mortem brains

Astrocytes were cultured from superior frontal gyrus of post-mortem brains donated to the Sun Health Research Institute Brain Program according to a protocol described previously (Lue et al., 2001b). Astrocytes were maintained in Dulbecco's Modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS).

IL-1β and interferon-γ (IFN-γ) (PeproTech, Rocky Hills, NJ) and recombinant $A\beta_{1-42}$ (rPeptide, Bogart, GA) were used to stimulate astrocytes for the study of $sPLA_2$-IIA mRNA expression. Lyophilized $A\beta_{1-42}$ were dissolved in 0.1 M NaOH and buffered with phosphate buffered saline to make a final concentration of 500 mM. The peptide solution was subsequently incubated at 37°C for 18 hours to promote oligomerization. Aliquots of the oligomerized $A\beta_{1-42}$ were stored in liquid nitrogen until experiments were performed. Twenty-four hours before treatments, culture media was exchanged for serum-free DMEM. Cells were then incubated in serum-free DMEM with IL-1β (20 ng/ml), IFN-γ (100 ng/ml), or 2.5 mM $A\beta_{1-42}$ for 24 h at 37°C. After incubation, cells were processed for RNA extraction.

RNA isolation, reverse transcription polymerase chain reaction (RT-PCR), and real time PCR

RNA was extracted from frozen brains and cultured astrocytes with Trizol reagent according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). RNA was isolated from hippocampus and cerebellum from 10 AD and 10 ND cases (Table 1). The integrity of isolated RNA was confirmed by denaturing agarose gel electrophoresis, and quantified by ultraviolet spectrophotometry. Total cellular RNA (1-2 μg) was reverse transcribed with random hexamers using Superscript III reverse transcriptase (Invitrogen, CA) as previously described (Xu et al., 2003; Walker et al., 2006).

RT-PCR was carried out to assess $sPLA_2$-IIA mRNA expression in astrocyte cultures. In this study, primers for *$sPLA_2$-IIA* are*:* forward 5'-GACTCATGACTGTTGTTACAACC-3' and reverse 5'-

TCTCAGGACTCTCTTAGGTACTA-3' that amplify a 493 bp fragment, and primers for *β-actin* are: forward 5'-TGGAGAAGAGCTATGAGCTGCCTG-3' and reverse 5'-GTGCCACCAGACAGCACTGTGTTG-3' that amplify a 289 bp fragment (Xu et al., 2003). After amplifications of 40 cycles for $sPLA_2$-IIA or 25 cycles for b-actin, a 5 μl aliquot of each reaction mixture was applied to 6% acrylamide gels. Bands were quantified using AlphaEaseFC software (Alpha Innotech, San Leandro, CA). Expression values were normalized for the levels of β-actin, which was used as the reference cellular transcript.

Table 2.1: Postmortem human brains used in the study of sPLA2-IIA expression

Cases	Clinical Diagnosis	Gender	Age (years)	PMI (hours)	Type of Study	Brain Region
1	ND	M	78	2.7	IHC	ITG
2	ND	M	81	2.7	IHC	I TG
3	ND	M	69	2.2	IHC	HPC, ITG
4	ND	M	84	2.5	IHC	HPC, ITG
5	ND	M	78	1.7	IHC	HPC, ITG
6	ND	M	94	3.0	RNA	HPC, CB
7	ND	M	85	3.2	RNA	HPC, CB
8	ND	F	85	2.5	RNA	HPC, CB
9	ND	F	86	2.0	RNA	HPC, CB
10	ND	F	88	3.0	RNA	HPC, CB
11	ND	F	94	2.5	RNA	HPC, CB
12	ND	F	86	2.5	RNA	HPC, CB,
13	ND	F	83	2.5	RNA	HPC, CB
14	ND	F	94	2.3	RNA	HPC, CB,
15	ND	F	78	2.8	IHC	ITG
					RNA	HPC, CB
16	ND	F	88	3.5	RNA	HPC, CB
17	AD	M	86	3.0	IHC	ITG
18	AD	M	87	3.0	IHC	HPC
					RNA	HPC, CB
19	AD	M	79	2.0	IHC	HPC
					RNA	HPC, CB
20	AD	M	94	3.8	IHC	ITG
21	AD	M	92	2.0	IHC	ITG
22	AD	F	89	3.0	IHC	HPC
					RNA	HPC, CB
23	AD	F	80	2.3	IHC	HPC, ITG
24	AD	F	85	1.7	RNA	HPC, CB
25	AD	F	95	3.2	RNA	HPC, CB
26	AD	F	91	3.0	RNA	HPC, CB
27	AD	F	89	2.3	RNA	HPC, CB
28	AD	F	97	1.5	IHC	ITG
29	AD	F	64	3.2	IHC	ITG
30	AD	F	77	2.8	IHC	ITG
31	AD	F	85	2.3	IHC	HPC
32	AD	F	90	3.0	RNA	HPC, CB

Abbreviations: ND: Non Demented Control, AD: Alzheimer's Disease, M: Male; F: Female; PMI: Post Mortem Interval; IHC: Immunohistochemistry; HPC: Hippocampus; ITG: Inferior Temporal Gyrus, CB: Cerebellum.

Real time PCR was used for determination of levels of $sPLA_2$-IIA mRNA in brain tissues. Taqman primers and probes specific for human $sPLA_2$-IIA and ribosomal 18S RNA were obtained from Applied Biosystems (Foster City, CA). For each sample (analyzed in triplicate), a pool containing Brilliant qPCR master mix (Stratagene, La Jolla, CA), Taqman probes, along with the cDNA was prepared, and then aliquoted into 96 well microtiter qPCR plates. Each analysis contained a series of diluted samples for standard curve purposes, as well as negative template and negative reverse transcriptase control samples. The real time PCR was carried out under optimized conditions using a Stratagene Mx3000p qPCR instrument. At the end of the run, relative expression results were calculated from the Ct values of each sample using the Mx3000p operating software. Each run was considered satisfactory if the standard curve covering a 1000-fold dilution range gave R^2 of > 0.98. Results were expressed relative to levels of 18S ribosomal RNA present in the samples, which were determined in the same manner.

Immunohistochemistry

Free-floating 20 μm sections from hippocampus and inferior temporal gyrus (ITG) were cut from 4% paraformaldehyde-fixed human brains and were used to study $sPLA_2$-IIA protein expression. Our previously published immunohistochemical procedure was used for this purpose (Walker et al., 2001). Sections were sequentially incubated with a monoclonal antibody to $sPLA_2$-IIA (Cayman, Ann Arbor, MI; 1:500 dilution, 18 hours, room temperature) in a phosphate buffered saline containing 0.3% Triton-X 100 (PBS-T). This was followed by reaction with biotinylated anti-mouse IgG

(Vector Laboratories, Burlingame, CA; 1:2000, 2 hours) and washed with PBS-T before applying avidin-biotin peroxidase complex (ABC) solution (Vector Laboratories, Burlingame CA; 1:2000, 1 hour). We detected bound antibody-antigen enzyme complex by reaction of sections with nickel-enhanced diaminobenzidine (DAB) solution (Lue et al., 2001b; Lue et al., 2001a). For two-color double immunohistochemistry, brain sections were first immunoreacted with nickel-DAB solution, then washed, and followed by 1% hydrogen peroxide to block peroxidase activity. Subsequently, sections were reacted with a polyclonal antibody to glial fibrillary acidic protein (GFAP; DAKO, Carpinteria, CA) to identify reactive astrocytes. Detection of GFAP was carried out using the same procedure described, with the exception that biotinylated anti-rabbit IgG and DAB substrate without nickel enhancement were used. These procedures produced $sPLA_2$-IIA immunoreactivity in dark blue color and GFAP in brown color. In some of the sections, an antibody to amyloid b (3D6, Elan Pharmaceuticals, South San Francisco, CA; 1:2000) was used to detect amyloid plaques. Some of the immunoreacted sections were counterstained with 1% neutral red to provide a general view of the cell populations in tissues. The mounted sections were dehydrated through graded ethanol and coverslipped with Permount embedding solution. The number of $sPLA_2$-IIA immunoreactive astrocytes associated with amyloid plaques was counted. Following double immunoreaction with $sPLA_2$-IIA and GFAP, sections were mounted and counter-stained with 1% thioflavin S (in 70% alcohol) for 15 minutes, dehydrated in 70% alcohol, and coverslipped with Vectashield mounting medium (Vector Laboratories, CA).

Quantifying $sPLA_2$-IIA-positive astrocytes in AD and ND brain sections

To estimate the percentage of $sPLA_2$-IIA-positive astrocytes, we used a semi-quantitative cell counting procedure with brain sections containing dentate gyrus (DG), CA3, or ITG that had been reacted with antibodies to detect $sPLA_2$-IIA and GFAP. In each brain region, the total number of GFAP immunoreactive cells and GFAP/$sPLA_2$-IIA immunoreactive cells were counted using a 1-mm^2 reticle, mounted in the eye-piece of an Olympus microscope, using 20X and 40X objective lenses (Olympus, Melville, NY). In the ITG sections, 10 vertical regions encompassing the width of the 1-mm^2 reticle field were counted. In each vertical region, counting began at the outer edge of the molecular layer and finished at the interface of the multiform layer and white matter. Cell counting was performed by a blinded examiner and in each vertical region mean cell numbers from 10 vertical fields were obtained. From this, we calculated the percentage of $sPLA_2$-IIA immunoreactive astrocytes in ITG for each case from 6 AD and 6 ND samples. In the CA3 region, we started counting at the CA3 boundary, and counted 5 consecutive, 1-mm^2 reticle fields covering the pyramidal cell layers. In the DG region, we began counting at the hilus and counted the 1-mm^2 reticle fields consecutively as far as the junction of the DG and CA region. The percentages of $sPLA_2$-IIA-positive astrocytes in the DG and CA3 regions were determined from 4 AD and 4 ND cases.

Using the same methodology, the number of $sPLA_2$-IIA-positive cells that co-localized with thioflavin S-positive plaques was counted. In each reticle field, thioflavin S-positive plaques were first visualized with a fluorescence microscope followed by phase contrast observation. Percentages of $sPLA_2$-IIA-positive astrocytes that co-localized with thioflavin S-positive plaques were obtained from the total number of $sPLA_2$-IIA-positive astrocytes.

Statistical analysis

Student's *t* test, or one-way ANOVA followed by Tukey posthoc multiple comparison test was used to analyze data using the GraphPad Prism 4 software. Significant differences between groups were assumed for P values < 0.05.

Results

Expression of sPLA$_2$-IIA mRNA in hippocampus and cerebellum of AD and ND brains

To demonstrate sPLA$_2$-IIA mRNA expression in human brain, we measured levels of sPLA$_2$-IIA mRNA by real time PCR analysis of RNA prepared from hippocampus and cerebellum samples from AD and ND patients. Hippocampal tissues for RNA purification were confined mainly to CA3 and dentate gyrus (DG) areas, as tissues from CA1 were not available. We detected a significant, 4.5-fold increase ($p<0.01$) in sPLA$_2$-IIA mRNA in AD hippocampus samples as compared to ND. On the other hand, there was no difference between sPLA$_2$-IIA mRNA levels in cerebellar samples from AD and ND brains.

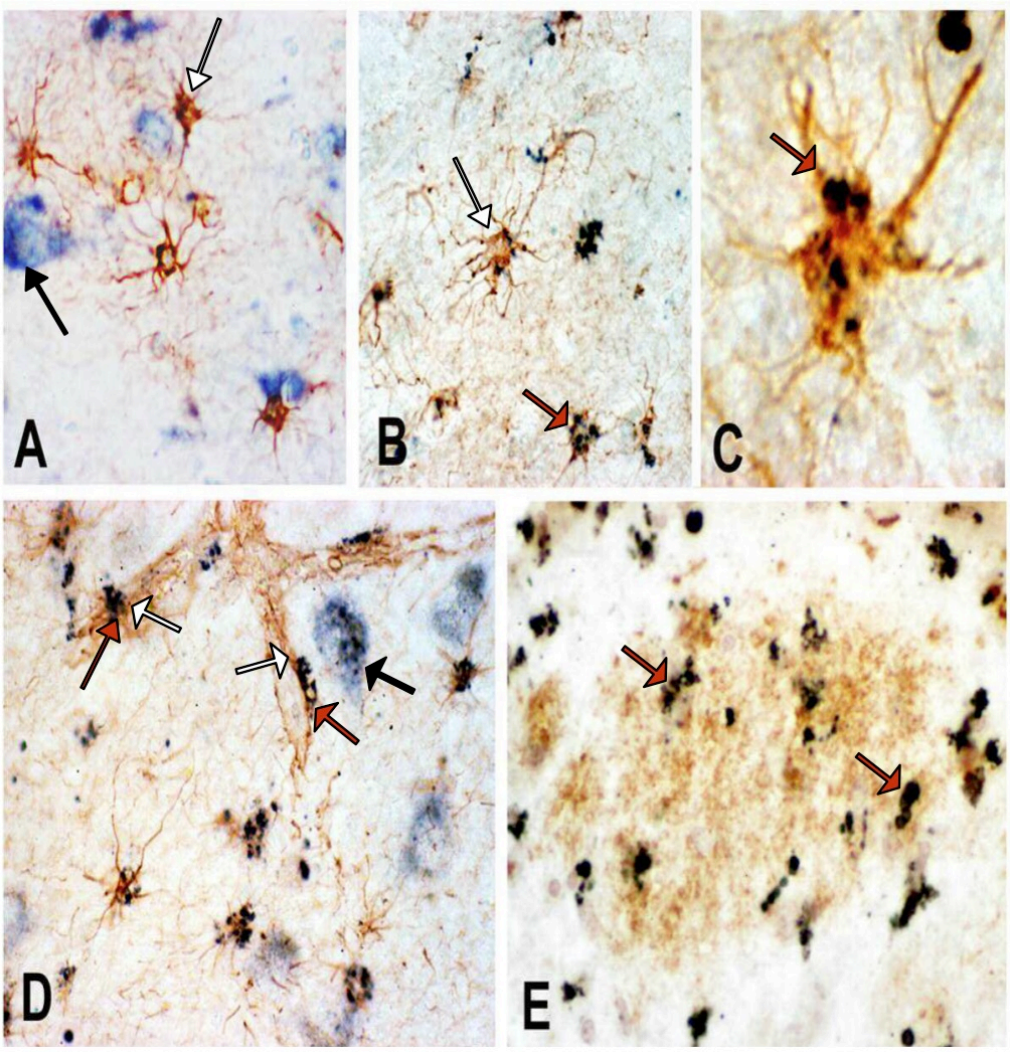

Figure 2.1 - sPLA2-IIA immunoreactivity in human postmortem brain tissues. Double immunostaining depicting sPLA2-IIA immunoreactivity in dark blue color and GFAP immunoreactivity in brown color is shown in panels A-D (using 20X and 40X objective lenses). Panel A demonstrates that little sPLA2-IIA immunoreactivity is present in a cluster of GFAP immunoreactive astrocytes in ND hippocampus. Panel B shows many GFAP-positive astrocytes (white arrow) labeled with intense immunoreactivity for sPLA2-IIA (dark immunoreactive products, red arrow) in AD hippocampus. At higher magnification (Panel C), sPLA2-IIA immunoreactivity is shown in an astrocyte cell body in granular-like structures (red arrow). Panel D shows that immunoreactivity for sPLA2-

IIA (red arrows) is also present in GFAP-positive astrocytes (white arrows) surrounding microvessels in AD hippocampus. We also detected sPLA2-IIA immunoreactivity in hippocampal neurons (black arrows) in ND (Panel A) and AD (panel B) hippocampus. In Panel E, several sPLA2-IIA immunoreactive profiles (red arrows) are co-localized with an amyloid plaque (brown immunoreactive area) detected by immunohistochemistry with an antibody to Aβ.

Increased immunoreactivity of sPLA$_2$-IIA in astrocytes of AD brain

Immunohistochemistry was used to demonstrate cell-associated sPLA$_2$-IIA protein in AD and ND brains. As shown in Figure 1A, there were few GFAP-positive astrocytes present in the hippocampal DG area from ND brain and these cells, which appeared to be forming astrocyte foot contacts with an amyloid plaque, showed little sPLA$_2$-IIA immunoreactivity. A higher number of GFAP-positive astrocytes and sPLA$_2$-IIA/GFAP-positive astrocytes were present in AD hippocampal regions (Fig. 1B and 1C). Immunoreactivity of sPLA$_2$-IIA was also detected in GFAP-positive cells lining the blood vessels (Fig. 1D), and co-localized with amyloid deposits (Fig. 1E).

To investigate whether sPLA$_2$-IIA-positive astrocytes are co-localized with amyloid deposits that contain Aβ in b-sheet conformation, brain sections double-immunoreacted with sPLA$_2$-IIA and GFAP were stained with thioflavin S fluorescence dye. Thioflavin S-positive plaques were present in the DG, CA3, and ITG of all AD cases; no thioflavin S-positive plaques were detected in the DG and CA3 regions of ND cases. Nevertheless, thioflavin S-positive plaques were present in the ITG of two ND cases. A sub-population of sPLA$_2$-IIA-positive astrocytes co-localized with thioflavin S-positive plaques in AD patients as demonstrated in the same brain sections that were processed for double immunohistochemistry for GFAP and sPLA$_2$-IIA antibodies (Fig. 2B) and for thioflavin S histochemistry (Fig. 2A).

We have quantified the percentages of astrocytes that were immunoreactive for sPLA$_2$-IIA and GFAP, and also the percentages of sPLA$_2$-IIA-positive astrocytes that are

associated with thioflavin S-positive plaques from brain sections containing DG, CA3, and ITG regions in AD and ND patients (see Table 1 for patient information). The results are shown in Table 2. Data show firstly that significantly greater percentages of GFAP-positive astrocytes were immunoreactive for $sPLA_2$-IIA in AD cases than in ND cases in all three brain regions. Secondly, in the gray matter of ITG, more than two thirds of $sPLA_2$-IIA-positive astrocytes in AD tissue sections co-localized with thioflavin S-positive plaques. Thirdly, among the three brain regions tested, the DG in AD brains contained the highest percentage of $sPLA_2$-IIA-positive astrocytes. However, the majority of the $sPLA_2$-IIA-positive astrocytes in the hippocampal regions were not associated with thioflavin S-positive plaques.

$sPLA_2$-IIA immunoreactivity was not detected in microglial cells (not shown); however, $sPLA_2$-IIA immunoreactivity was observed in neurons (identified based on their morphology) in both ND and AD brains (Fig. 1A and 1D). Unlike the immunostaining for astrocytes, which showed punctate dark spots, $sPLA_2$-IIA immunoreactivity in neurons shows an amorphous distribution pattern.

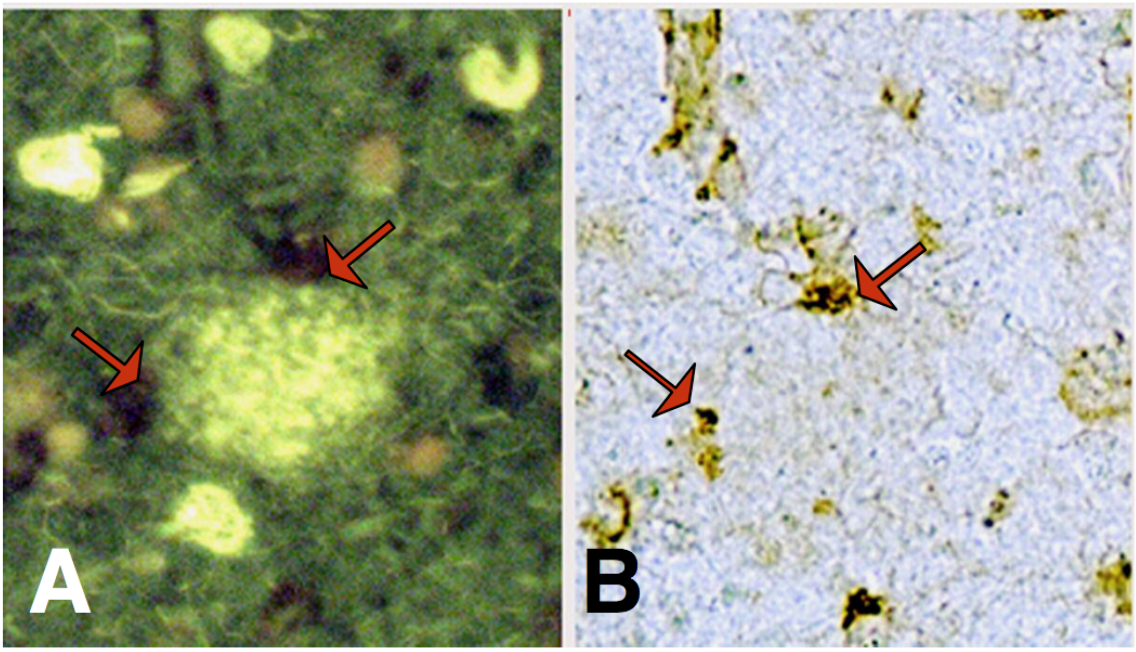

Figure 2.2 – Co-localization of sPLA2-IIA-positive astrocytes with thioflavin S-positive plaques. Double immunostaining of sPLA2-IIA and GFAP combined with thioflavin S staining shows the presence of sPLA2-IIA (red arrows) in GFAP-positive astrocytes (panels A and B) and their association with thioflavin S-positive amyloid plaques (green fluorescent area in panel A) in an ITG section from an AD case.

Table 2.2: sPLA2-IIA-positive astrocytes in hippocampus and inferior temporal gyrus of Alzheimer (AD) and nondemented (ND) subjects.

Brain region	**Dentate gyrus**		**CA3 region**		**Inferior temporal gyrus**	
Subjects	**AD**	**ND**	**AD**	**ND**	**AD**	**ND**
Total $sPLA_2$-IIA-positive astrocytes	50.82 ± 9.00[1]***	1.27 ± 0.96	24.11 ± 5.15***	0.00	12.86 ± 2.90***	1.99 ± 0.56
Plaque-associated $sPLA_2$-IIA-positive astrocytes[2]	0.66 ± 0.21*	0.00	1.59 ± 0.38**	0.00	8.60 ± 2.74*	0.51 ± 0.35

[1]Astrocyte counts are given as percent of all GFAP-positive astrocytes. Values are expressed as mean ± SD.
[2]Plaque-associated astrocytes were identified by co-staining with thioflavin S
*, **, ***Value is significantly different from corresponding ND value (Student's *t* test): *$p < 0.01$; **$p < 0.005$; ***$p < 0.001$

Pro-inflammatory cytokines and Ab_{1-42} induce $sPLA_2$-IIA mRNA in human astrocytes

To further demonstrate expression and regulation of $sPLA_2$-IIA in astrocytes, human astrocytes cultured from superior frontal gyrus of post-mortem AD brains were treated with Ab_{1-42} (2.5mM), IL-1b (20 ng/ml), and IFNg (100 ng/ml), alone or in combination for 24 hours. When stimulated with IL-1b, astrocytes from AD post-mortem brain developed reactive morphology with slender long processes as compared to untreated astrocytes (Fig. 3A and 3B). RT-PCR indicated very low $sPLA_2$-IIA mRNA expression in control and IFNγ-treated astrocytes (Fig. 3C and 3D), but significant increases were observed upon stimulating astrocytes with $A\beta_{1-42}$ and IL-1β. When $A\beta_{1-42}$ and IL-1β were given together, there was no further enhancement of $sPLA_2$-IIA mRNA expression, compared to each treatment alone.

Discussion

In this study, we characterize the expression of $sPLA_2$-IIA in AD and ND brains. In AD, severe pathological changes occur, topographically and quantitatively, in the hippocampus and temporal cortical areas, whereas cerebellum is relatively spared from AD pathology. Using real time PCR for measuring $sPLA_2$-IIA mRNA in hippocampus and cerebellum, we showed a significant increase in $sPLA_2$-IIA mRNA in the hippocampus of AD brains as compared to ND brains, whereas no increase was observed in cerebellum. Using immunohistochemistry, we demonstrated that GFAP-positive astrocytes are the main cell type that express $sPLA_2$-IIA protein. In hippocampus and ITG, the percentages of astrocytes that expressed $sPLA_2$-IIA protein are significantly higher in the AD brains when compared to ND brains. This is the first demonstration of upregulation of $sPLA_2$-IIA

protein in astrocytes in AD brains. The increase in $sPLA_2$-IIA expression in AD hippocampus, but not in AD cerebellum, is in agreement with the neuropathological observations that reactive astrocytes are increasingly associated with pathology in hippocampus and cortex, whereas diffuse amyloid deposits and limited astrocyte activation are found in cerebellum (Joachim et al., 1989; Pike et al., 1995).

It has been established that the number of GFAP-positive astrocytes associated with amyloid plaques changes during plaque formation. There are fewer GFAP-positive astrocytes associated with diffuse plaques; while more are associated with neuritic plaques containing fibrillar Aβ and dystrophic neuritis (Mrak et al., 1996). Thioflavin S fluorescence dye can detect amyloid fibrils in β-pleated sheet formation, a state of aggregation that occurs when diffuse plaques progress to neuritic plaques. Although thioflavin S-positive plaques are more abundant in AD brains, there are occasionally such plaques in the neocortex of normal aging brains (Morris et al., 1996; Haroutunian et al., 1998). In this study, thioflavin S-positive plaques were observed in ITG in 2 ND patients. We analyzed whether increases in the number of $sPLA_2$-IIA-positive astrocytes are associated with thioflavin S-positive plaques. Our results indicated that these cells were highly associated with thioflavin S-positive plaques in ITG sections, but not in DG or CA3 regions of the hippocampus. In the ITG of ND brains, a very low percentage of $sPLA_2$-IIA-positive astrocytes is present in the thioflavin S-positive plaques. These data suggest that the induction of $sPLA_2$-IIA protein in astrocytes could result from their interaction with Ab and other inflammatory stimuli. This notion is supported by data obtained from experiments using astrocyte cultures derived from post-mortem human brains. Since the IL-1b signaling pathway is considered a key pathway for induction of pro-inflammatory

molecules in brain (Moynagh, 2005), it is possible that a progressive elevation of IL-1b in AD brain could lead to persistent upregulation of inflammatory proteins including $sPLA_2$-IIA in astrocytes (Samuelsson et al., 2005). Results from astrocyte cultures showed significant induction of $sPLA_2$-IIA mRNA by IL-1β or by Aβ alone. These results are in agreement with our previous studies with rat astrocytes (Li et al., 1999; Xu et al., 2003). Because IL-1β secreted by activated microglia is involved in initiating astrocyte activation and inflammatory cascade (Griffin and Mrak, 2002), its ability to induce $sPLA_2$-IIA mRNA in astrocytes suggests that $sPLA_2$-IIA upregulation could be engaged in early inflammatory events resulting from astrocyte activation. Taken together, these results are in agreement with the ability of pro-inflammatory cytokines and Aβ to mediate inflammatory responses in astrocytes including the induction of $sPLA_2$-IIA.

The apparent lack of $sPLA_2$-IIA immunoreactivity in microglial cells seems to be in agreement with our earlier study with a rat stroke model in which up-regulation of $sPLA_2$-IIA immunoreactivity was observed primarily in reactive astrocytes but not in microglia (Lin et al., 2004). Wang et al. (Wang et al., 2005) also demonstrated the ability of lipopolysaccharide (LPS) to stimulate and release sPLA2-IIA from astrocytes but not from microglial cells. Results in this study also show immunoreactivity of $sPLA_2$-IIA in hippocampal neurons with intensity and staining patterns that are different from those in astrocytes. Since this staining pattern appears in all neurons in both ND and AD samples, more studies are needed to characterize this immunoreactivity. $sPLA_2$-IIA immunoreactivity has also been reported in neurons from other brain regions, including Purkinje neurons of rat cerebellum (Shirai and Ito, 2004). Aside from $sPLA_2$-IIA, other types of $sPLA_2$ with similar structure, e.g., groups 1B, IIE, V and X, are present in distinct

brain regions (Macchioni et al., 2004; Kolko et al., 2006). Consequently, the functional role of different $sPLA_2$ in neurons and glia, and the specific subtypes induced in response to injury, remain an important area to be further explored.

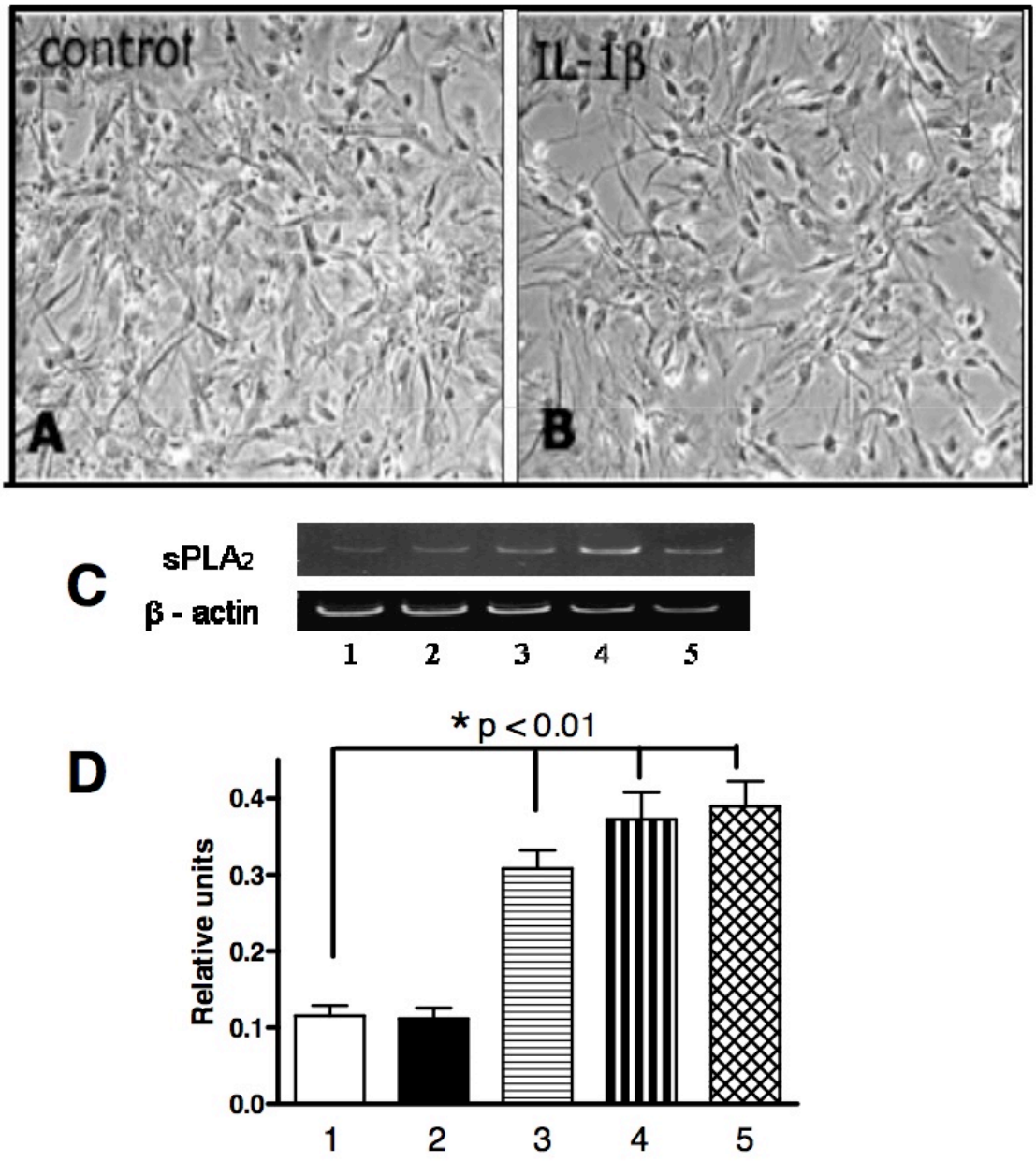

Figure 2.3 – Induction of sPLA2-IIA mRNA expression by cytokines and A-beta(1-42) in cultured human astrocytes. Phase contrast micrographs show human astrocytes in control (panel A) and IL-1β-stimulated cultures (panel B) for 24 hours. Human post-mortem astrocytes were used for the sPLA2-IIA RNA study. Experiments were performed using cultures derived from 3 neuropathologically confirmed AD cases. A representative gel depicting PCR-amplified fragments for sPLA2-IIA and beta-actin is shown in panel C. Gel

lanes 1-5 represent the following treatments used in the astrocyte cultures: 1. Control; 2. IFN-γ (100 ng/ml); 3. Aβ(1-42) (2.5 μM); 4. IL-1β (20 ng/ml); 5. IL-1β and Aβ(1-42). Twenty-four hours after treatment, RNA was extracted from cells, reverse transcribed, and RT-PCR was carried out as described in methods. Panel D shows a bar graph depicting relative units of sPLA2-IIA expression after normalization with beta-actin. Significant differences (*) comparing treatment groups with controls were obtained by one-way ANOVA followed by Tukey multiple comparison post hoc test.

Secretory PLA_2-IIA has been regarded as an inflammatory protein in the periphery and is upregulated in a number of cardiovascular diseases (Aarsman et al., 2000; Hurt-Camejo et al., 2001; Touqui and Alaoui-El-Azher, 2001). The physiological consequences of inflammatory factors released from glial cells and their ability to damage neurons have been a topic of intense investigation. Our earlier study with astrocytes has demonstrated a role for $sPLA_2$-IIA induced by pro-inflammatory cytokines in the production of prostaglandins (Xu et al., 2003). Other studies have also shown that secreted $sPLA_2$-IIA can perturb cellular membranes, especially those undergoing apoptosis (Atsumi et al., 1997; Fourcade et al., 1998; Nijmeijer et al., 2003). In PC12 cells, lysophospholipids produced by $sPLA_2$-IIA were shown to alter neurite outgrowth (Ikeno et al., 2005). Furthermore, $sPLA_2$ from bee venom was shown to modulate the activities of ionotropic glutamate receptors and Ca^{2+} channels, resulting in neuronal excitotoxicity and apoptosis (Kolko et al., 2002; Yagami et al., 2004). Due to the possible damaging effects of $sPLA_2$-IIA on neuronal function, there is strong rationale to develop specific inhibitors for this enzyme (Yagami et al., 2002a). CHEC-9, a peptide inhibitor of $sPLA_2$-IIA, was shown to ameliorate PLA_2-directed inflammation in both acute and chronic neurodegenerative disease models (Cunningham et al., 2004). Our data demonstrating $sPLA_2$-IIA as a new inflammatory factor for AD may further facilitate the development of novel therapeutics to retard the progression of this disease.

Conclusion

This study demonstrates for the first time an increase in protein expression of $sPLA_2$-IIA in GFAP-positive astrocytes in AD brains as compared to ND brains. The

ability of pro-inflammatory cytokines and $A\beta_{1-42}$ to induce $sPLA_2$-IIA mRNA in astrocytes further supports a possible role for $sPLA_2$-IIA in the inflammatory responses in AD.

List of abbreviations used: AA, arachidonic acid; Aβ, amyloid beta; AD, Alzheimer's disease; $cPLA_2$, cytosolic PLA_2; DAB, diaminobenzidine; DG, dentate gyrus; DMEM, Dulbecco's Modified Eagle Medium; FBS, fetal bovine serum; IFN-γ, interferon-γ; IL-1β, interleukin-1β; ITG, inferior temporal gyrus; GFAP, glial fibrillary acidic protein; ND, non-demented; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PLA_2, phospholipase A_2; $sPLA_2$, secretory phospholipase A_2.

Competing interests: The authors declare that they have no competing interests.

Authors' contributions: GSDM, LL and DGW acquired samples, performed all of the immunohistochemical studies and PCR analyses of $sPLA_2$-IIA mRNA expression in human brains and cultured astrocytes, and edited the manuscript. MDJ, AYS, AS and GYS participated in the design and coordination of the studies and helped to draft the manuscript. GYS, LL, and DGW provided the funding for the project. All authors read and approved the final manuscript.

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REFERENCES:

Aarsman AJ, Neys FW, van der Helm HA, Kuypers FA, van den Bosch H (2000) Sera of patients suffering from inflammatory diseases contain group IIA but not group V phospholipase A(2). Biochim Biophys Acta 1502:257-263.

Adibhatla RM, Hatcher JF (2006) Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radical Biol Med 40:376-387.

Akama KT, Albanese C, Pestell RG, Van Eldik LJ (1998) Amyloid beta-peptide stimulates nitric oxide production in astrocytes through an NFkappaB-dependent mechanism. Proc Natl Acad Sci U S A 95:5795-5800.

Akiyama H et al. (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383-421.

Atsumi G, Murakami M, Tajima M, Shimbara S, Hara N, Kudo I (1997) The perturbed membrane of cells undergoing apoptosis is susceptible to type II secretory phospholipase A2 to liberate arachidonic acid. Biochim Biophys Acta 1349:43-54.

Balboa MA, Varela-Nieto I, Killermann Lucas K, Dennis EA (2002) Expression and function of phospholipase A(2) in brain. FEBS Lett 531:12-17.

Bazan NG (2003) Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. J Lipid Res 44:2221-2233.

Bazan NG (2005) Synaptic signaling by lipids in the life and death of neurons. Mol Neurobiol 31:219-230.

Cacquevel M, Lebeurrier N, Cheenne S, Vivien D (2004) Cytokines in neuroinflammation and Alzheimer's disease. Curr Drug Targets 5:529-534.

Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ (2002) Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J Neurosci Res 70:462-473.

Craft JM, Watterson DM, Van Eldik LJ (2006) Human amyloid beta-induced neuroinflammation is an early event in neurodegeneration. Glia 53:484-490.

Cunningham TJ, Souayah N, Jameson B, Mitchell J, Yao L (2004) Systemic treatment of cerebral cortex lesions in rats with a new secreted phospholipase A2 inhibitor. J Neurotrauma 21:1683-1691.

Farooqui AA, Yang HC, Rosenberger TA, Horrocks LA (1997) Phospholipase A2 and its role in brain tissue. J Neurochem 69:889-901.

Fourcade O, Le Balle F, Fauvel J, Simon MF, Chap H (1998) Regulation of secretory type-II phospholipase A2 and of lysophosphatidic acid synthesis. Adv Enzyme Regul 38:99-107.

Griffin WS (2006) Inflammation and neurodegenerative diseases. Am J Clin Nutr 83:470S-474S.

Griffin WS, Mrak RE (2002) Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. J Leukoc Biol 72:233-238.

Haroutunian V, Perl DP, Purohit DP, Marin D, Khan K, Lantz M, Davis KL, Mohs RC (1998) Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. Arch Neurol 55:1185-1191.

Hashmi N, Lee S, Cummings MP (2004) Abstracting Workflows: Unifying Bioinformatics Task Conceptualization and Specification Through Semantic Web Services. In, pp 1-7. Cambridge, Massachusetts USA.

Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ (1998) Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. Brain Res 785:195-206.

Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P (2001) Phospholipase A(2) in vascular disease. Circ Res 89:298-304.

Ikeno Y, Konno N, Cheon SH, Bolchi A, Ottonello S, Kitamoto K, Arioka M (2005) Secretory phospholipases A2 induce neurite outgrowth in PC12 cells through lysophosphatidylcholine generation and activation of G2A receptor. J Biol Chem 280:28044-28052.

Jefferies WA, Food MR, Gabathuler R, Rothenberger S, Yamada T, Yasuhara O, McGeer PL (1996) Reactive microglia specifically associated with amyloid plaques in Alzheimer's disease brain tissue express melanotransferrin. Brain Res 712:122-126.

Joachim CL, Morris JH, Selkoe DJ (1989) Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am J Pathol 135:309-319.

Kennedy BP, Payette P, Mudgett J, Vadas P, Pruzanski W, Kwan M, Tang C, Rancourt DE, Cromlish WA (1995) A natural disruption of the secretory group II phospholipase A2 gene in inbred mouse strains. J Biol Chem 270:22378-22385.

Kolko M, de Turco EB, Diemer NH, Bazan NG (2002) Secretory phospholipase A2-mediated neuronal cell death involves glutamate ionotropic receptors. Neuroreport 13:1963-1966.

Kolko M, Christoffersen NR, Barreiro SG, Miller ML, Pizza AJ, Bazan NG (2006) Characterization and location of secretory phospholipase A2 groups IIE, V, and X in the rat brain. J Neurosci Res.

Li W, Xia J, Sun GY (1999) Cytokine induction of iNOS and sPLA2 in immortalized astrocytes (DITNC): response to genistein and pyrrolidine dithiocarbamate. J Interferon Cytokine Res 19:121-127.

Lin TN, Wang Q, Simonyi A, Chen JJ, Cheung WM, He YY, Xu J, Sun AY, Hsu CY, Sun GY (2004) Induction of secretory phospholipase A2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. J Neurochem 90:637-645.

Lue LF, Walker DG, Rogers J (2001a) Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. Neurobiol Aging 22:945-956.

Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM, Jr., Brachova L, Yan SD, Walker DG, Shen Y, Rogers J (2001b) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35:72-79.

Macchioni L, Corazzi L, Nardicchi V, Mannucci R, Arcuri C, Porcellati S, Sposini T, Donato R, Goracci G (2004) Rat brain cortex mitochondria release group II secretory phospholipase A(2) under reduced membrane potential. J Biol Chem 279:37860-37869.

McGeer EG, McGeer PL (2003) Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 27:741-749.

Morris JC, Storandt M, McKeel DW, Jr., Rubin EH, Price JL, Grant EA, Berg L (1996) Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology 46:707-719.

Moynagh PN (2005) The interleukin-1 signalling pathway in astrocytes: a key contributor to inflammation in the brain. J Anat 207:265-269.

Mrak RE, Griffin WS (2005) Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 26:349-354.

Mrak RE, Sheng JG, Griffin WS (1996) Correlation of astrocytic S100 beta expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. J Neuropathol Exp Neurol 55:273-279.

Murakami M, Kudo I (2002) Phospholipase A2. J Biochem (Tokyo) 131:285-292.
Niessen HW, Krijnen PA, Visser CA, Meijer CJ, Erik Hack C (2003) Type II secretory phospholipase A2 in cardiovascular disease: a mediator in atherosclerosis and ischemic damage to cardiomyocytes? Cardiovasc Res 60:68-77.

Nijmeijer R, Willemsen M, Meijer CJ, Visser CA, Verheijen RH, Gottlieb RA, Hack CE, Niessen HW (2003) Type II secretory phospholipase A2 binds to ischemic flip-flopped cardiomyocytes and subsequently induces cell death. Am J Physiol Heart Circ Physiol 285:H2218-2224.

Pike CJ, Cummings BJ, Cotman CW (1995) Early association of reactive astrocytes with senile plaques in Alzheimer's disease. Exp Neurol 132:172-179.

Ranaivo HR, Craft JM, Hu W, Guo L, Wing LK, Van Eldik LJ, Watterson DM (2006) Glia as a therapeutic target: selective suppression of human amyloid-beta-induced upregulation of brain proinflammatory cytokine production attenuates neurodegeneration. J Neurosci 26:662-670.

Samuelsson M, Fisher L, Iverfeldt K (2005) beta-Amyloid and interleukin-1beta induce persistent NF-kappaB activation in rat primary glial cells. Int J Mol Med 16:449-453.

Shirai Y, Ito M (2004) Specific differential expression of phospholipase A2 subtypes in rat cerebellum. J Neurocytol 33:297-307.

Silvestrelli G, Lanari A, Parnetti L, Tomassoni D, Amenta F (2006) Treatment of Alzheimer's disease: From pharmacology to a better understanding of disease pathophysiology. Mech Ageing Dev 127:148-157.

Stephenson D, Rash K, Smalstig B, Roberts E, Johnstone E, Sharp J, Panetta J, Little S, Kramer R, Clemens J (1999) Cytosolic phospholipase A2 is induced in reactive glia following different forms of neurodegeneration. Glia 27:110-128.

Stephenson DT, Lemere CA, Selkoe DJ, Clemens JA (1996) Cytosolic phospholipase A2 (cPLA2) immunoreactivity is elevated in Alzheimer's disease brain. Neurobiol Dis 3:51-63.

Streit WJ (2004) Microglia and Alzheimer's disease pathogenesis. J Neurosci Res 77:1-8.

Stuchbury G, Munch G (2005) Alzheimer's associated inflammation, potential drug targets and future therapies. J Neural Transm 112:429-453.

Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 45:205-213.

Sun GY, Xu J, Jensen MD, Yu S, Wood WG, Gonzalez FA, Simonyi A, Sun AY, Weisman GA (2005) Phospholipase A2 in astrocytes: responses to oxidative stress, inflammation, and G protein-coupled receptor agonists. Mol Neurobiol 31:27-41.

Taketo MM, Sonoshita M (2002) Phospolipase A2 and apoptosis. Biochim Biophys Acta 1585:72-76.

Thwin MM, Ong WY, Fong CW, Sato K, Kodama K, Farooqui AA, Gopalakrishnakone P (2003) Secretory phospholipase A2 activity in the normal and kainate injected rat brain, and inhibition by a peptide derived from python serum. Exp Brain Res 150:427-433.

Touqui L, Alaoui-El-Azher M (2001) Mammalian secreted phospholipases A2 and their pathophysiological significance in inflammatory diseases. Curr Mol Med 1:739-754.

Valentin E, Lambeau G (2000) Increasing molecular diversity of secreted phospholipases A(2) and their receptors and binding proteins. Biochim Biophys Acta 1488:59-70.

Walker DG, Lue LF (2005) Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases. J Neurosci Res 81:412-425.

Walker DG, Lue LF, Beach TG (2001) Gene expression profiling of amyloid beta peptide-stimulated human post-mortem brain microglia. Neurobiol Aging 22:957-966.

Walker DG, Link J, Lue LF, Dalsing-Hernandez JE, Boyes BE (2006) Gene expression changes by amyloid {beta} peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes. J Leukoc Biol 79:596-610.

Wang G, Daniel BM, DeCoster MA (2005) Role of nitric oxide in regulating secreted phospholipase A2 release from astrocytes. Neuroreport 16:1345-1350.

Xu J, Chalimoniuk M, Shu Y, Simonyi A, Sun AY, Gonzalez FA, Weisman GA, Wood WG, Sun GY (2003) Prostaglandin E2 production in astrocytes: regulation by cytokines, extracellular ATP, and oxidative agents. Prostaglandins Leukot Essent Fatty Acids 69:437-448.

Yagami T, Ueda K, Sakaeda T, Itoh N, Sakaguchi G, Okamura N, Hori Y, Fujimoto M (2004) Protective effects of a selective L-type voltage-sensitive calcium channel blocker, S-312-d, on neuronal cell death. Biochem Pharmacol 67:1153-1165.

Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Takasu N, Tanaka K, Gemba T, Hori Y (2002) Human group IIA secretory phospholipase A2 induces neuronal cell death via apoptosis. Mol Pharmacol 61:114-126.

Chapter 3

INVOLVEMENT OF OXIDATIVE PATHWAYS IN CYTOKINE-INDUCED SECRETORY PHOSPHOLIPASE A2-IIA IN ASTROCYTES

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ABSTRACT

Recent studies have suggested the involvement of secretory phospholipase A2-IIA (sPLA2-IIA) in neuroinflammatory diseases. Although sPLA2-IIA is transcriptionally induced through the NF-κB pathway by pro-inflammatory cytokines, whether this induction pathway is affected by other intracellular signaling pathways has not been investigated in detail. In this study, we demonstrated the induction of sPLA2-IIA mRNA and protein expression in astrocytes by cytokines and detected the protein in the culture medium after stimulation. We further investigated the effects of oxidative pathways and botanical antioxidants on the induction pathway and observed that IL-1β-induced sPLA2-IIA mRNA expression in astrocytes is dependent on ERK1/2 and PI-3 kinase, but not p38 MAPK. In addition to apocynin, a known NADPH oxidase inhibitor, botanical antioxidants, such as resveratrol and epigallocatechin gallate, also inhibited IL-1β-induced sPLA2-IIA mRNA expression. These compounds also suppressed IL-1β-induced ERK1/2 activation and translocation of the NADPH oxidase subunit p67 phox from cytosol to membrane fraction. Taken together, these results support the involvement of reactive oxygen species from NADPH oxidase in cytokine induction of sPLA2-IIA in astrocytes and promote the use of botanical antioxidants as protective agents for inhibition of inflammatory responses in these cells.

1. **INTRODUCTION**

Phospholipases A2 (PLA2, EC3.1.1.4.) catalyze the hydrolysis of *sn*-2 fatty acids from phospholipids. There are more than 20 distinct mammalian isoforms of PLA2 belonging to the calcium-dependent cytosolic group IV PLA2 (cPLA2), the calcium-independent group VI PLA2 (iPLA2), or the small molecular weight group II secretory PLA2 (sPLA2) (Murakami et al., 1997; Sun et al., 2004; Sun et al., 2007; Burke and Dennis, 2008a, b). These enzymes are widely expressed across mammalian cell types and besides playing a role in maintaining integrity of phospholipids in the cell membrane, they are also involved in the production of arachidonic acid, a precursor for prostanoids.

Among more than 12 isoforms of sPLA2, considerable attention has been given to the sPLA2-IIA and inhibitors for this type of PLA2 (Boilard et al., 2006; Lambeau and Gelb, 2008; Oslund et al., 2008; Ibeas et al., 2009). This enzyme is a mediator connecting innate and adaptive immunity and is up-regulated in a number of coronary artery diseases, including atherosclerosis, sepsis, arthritis and infection (Leitinger et al., 1999; Tietge et al., 2005; Krijnen et al., 2006; Mallat et al., 2007; Kimura-Matsumoto et al., 2008; Ibeas et al., 2009). Up-regulation of sPLA2-IIA mRNA expression and immunoreactivity has been reported in rat brain after cerebral ischemia (Lin et al., 2004; Adibhatla and Hatcher, 2007) and in human Alzheimer's disease brain (Moses et al., 2006). Furthermore, in vitro studies demonstrated the critical role of sPLA2-IIA in neuronal channels and activity (Kolko et al., 2002; Yagami et al., 2002a; Mathisen et al., 2007).

Our earlier studies with cultured astrocytes provided evidence for the induction of sPLA2-IIA by inflammatory cytokines, such as interleukin 1-β (IL-1β) and tumor

necrosis factor alpha (TNF-α) (Sun and Hu, 1995; Xu et al., 2003a). Subsequent studies indicated that sPLA2-IIA is transcriptionally induced by pro-inflammatory cytokines through the NF-κB pathway (Sun and Hu, 1995; Tong et al., 1999; Lappas et al., 2004; Jaulmes et al., 2005). Nevertheless, whether transcriptional synthesis of sPLA2-IIA is regulated by other signaling cascades has not been explored in sufficient detail.

Recent studies provided evidence that a phagocyte-like NADPH oxidase, capable of generating reactive oxygen species (ROS) in the form of superoxide, is functionally active in astrocytes (Noh and Koh, 2000; Abramov et al., 2005; Liu et al., 2005). This NADPH oxidase contains both membrane and cytoplasmic components, and its activation has been linked to a number of cell surface receptors and signaling cascades (Bedard and Krause, 2007). In this study, we examined the involvement of the NADPH oxidase and other oxidative signaling pathways in cytokine induction of sPLA2-IIA in astrocytes. In addition, we tested the effects of the botanical antioxidants resveratrol and epigallocatechin gallate (EGCG) on sPLA2-IIA expression.

2. MATERIALS AND METHODS

2.1. Materials

Apocynin, anti-β-actin antibody and BSA were purchased from Sigma (St. Louis, MO, USA). Cytokines were purchased from R & D Systems (Minneapolis, MN, USA). SB203580 and U0126 and LY294002 were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Trizol, and Superscript III One Step RT-PCR kit were purchased from Invitrogen (Eugene, OR, USA). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA, USA). DITNC (immortalized rat

astrocytes) were obtained from ATCC (Rockville, MD, USA). Dulbecco's modified Eagle's medium (DMEM), DMEM:F12 1:1, and TryPLE (trypsin) were purchased from GIBCO-BRL (Gaithersburg, MD, USA). Polyclonal antibodies for sPLA2-IIA were obtained from BioVendor (Candler, NC, USA). Anti-phospho-ERK1/2 and anti-ERK1/2 were obtained from Cell Signaling Technology (Danvers, MA, USA), and p67 phox antibodies were obtained from Upstate (Billerica, MA, USA). SuperSignal West Pico chemiluminescence was purchased from Pierce (Rockford, IL, USA).

2.2. Cell culture

The immortalized rat astrocyte cell line (DITNC) was maintained in DMEM with 10% FBS and 1% penicillin/streptomycin (P/S) at 37 ^{o}C with 5% CO2 and 95% humidity. Prior to experiments, cells were starved for 4 hours in DMEM medium, followed by treatments with different conditions as described. Polyphenols and inhibitors were added to the cell medium 1 h before treatment with cytokines for 18 hours.

2.3. Crude membrane preparation

In experiments requiring preparation of crude membrane fractions, astrocytes were pretreated with resveratrol or EGCG followed by exposing to IL-1β for 10 min. Cells were then suspended in 0.25 M sucrose, 5 mM $MgCl_2$, 2 mM EGTA, 2 mM EDTA, 10 mM Tris (pH 7.5), 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml pepstatin, and 10 μg/ml aprotinin and were disrupted by brief sonication and centrifuged at 1000×*g* for 5 min at 4 °C to remove unbroken cells and nuclei. Supernatant was removed and centrifuged at 100,000×*g* for 1 h at 4 °C (SW40 rotor, Beckman

ultracentrifuge). The membrane pellet was resuspended in buffer with 0.5% (v/v) Triton X-100 for 1 h at 4 °C (Min et al., 2004) and expression of p67 phox protein was analyzed by Western blot.

2.4. Western blot analysis

Astrocytes were cultured in 60 mm or 100 mm dishes until 90% confluent. After treatment, cells were washed with ice-cold PBS twice, followed by lysing with lysis buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 100 mM NaCl, 0.1% SDS, 1 mM PMSF, 1 mM sodium orthovanadate, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 10 μg/ml aprotinin). Protein concentrations were determined by the Bradford assay (Bradford, 1976). Equivalent amounts of protein for each sample were resolved in a 15% SDS-Page and run for 60 minutes at 200 V. Proteins were subsequently transferred at 100 V for 1 h to nitrocellulose membranes. Membranes were incubated in Tris-buffered saline, pH 7.4, with 0.5% Tween 20 (TBS-T) containing 5% nonfat milk for 1 h at room temperature. Blots were reacted with the primary antibody (TBS-T with 5% milk) at 4 ^{o}C overnight, washed 3 times for 5 min in TBS-T, and then incubation with the secondary antibody (TBS-T with 5% milk) for 1 h at room temperature. After washing 3 times for 5 min with TBS-T, SuperSignal West Pico chemiluminescence reagents from Pierce were used to signal detection. Band density was measured using Quantity One software (Bio-Rad, Hercules, CA, USA). In some studies, the blots were striped using the standard protocol, washed and re-probed with anti-β-actin. The following antibody concentrations or dilutions were used: sPLA2-IIA (1: 1000), phospho-ERK1/2 (1:1000), ERK1/2 (1:2000), p67 phox (1:1000), β-actin (1:30000) secondary antibodies (1:5000).

2.5. Semi-quantitative RT-PCR

Cells were washed with PBS following treatment and RNA was isolated with Trizol (Invitrogen) according to manufacturer's instructions. A SuperScript III One-Step kit (Invitrogen) was used according to manufacturer's instructions. Briefly, one microgram of RNA was used in the one-step RT-PCR with 50 pmol of the following oligonucleotide primers designed from rat gene sequences for sPLA2-IIA: sense 5′-TGACTCATGACTGTTGTTACAACC-3′ and antisense 5′-TCTCAGGACTCTCTTAGGTACTA-3′ (amplifies a 493 bp fragment); and for β-actin: sense 5′-TGGAGAAGAGCTATGAGCTGCCTG-3′ and antisense 5′-GTGCCACCAGACAGCACTGTGTTG-3′ (amplifies a 289 bp fragment). Reverse transcription at 50°C for 30 min and inactivation of reverse transcriptase at 94 °C for 2 min were followed by 40 cycles of amplification for sPLA2-IIA cDNA (annealing at 55 °C for 1 min) or 30 cycles of amplification for β-actin cDNA (annealing at 55°C for 1 min). Each cycle included a 15 s denaturation step at 94 °C, an annealing step, and a 1 min extension step at 68 °C. A 5 min extension at 68 °C was carried out at the end of the final cycle. Amplified DNA was visualized on a 2% agarose gel with ethidium bromide and analyzed with Quantity One software.

2.6. Reverse transcription

Reverse transcription of RNA was carried out using the Taqman Reverse Transcription Kit following the manufacturer's instructions (Ambion, Austin, TX, USA). Briefly, RNA concentration was determined with spectrophotometry at 260 nm and concentrations of samples were normalized to 30 μg/ml. Oligo $d(T)_{16}$ was combined with other reaction components, and 6.15 μl of reaction mix was added to each tube

containing 3.85 µl of RNA for a total volume of 10 µl. The samples were incubated at 25 ^{o}C for 10 min, followed by the reverse transcription step at 48 ^{o}C for 30 min, and reverse transcriptase inactivation at 95 ^{o}C for 5 min.

2.7. Quantitative real-time PCR

The ABI 7300 Taqman by Applied Biosystems was used for real-time PCR according to manufacturer's instructions. A pre-designed primer and probe set was purchased for determination of expression of sPLA2-IIA (Assay ID Rn00580999) based on NCBI sequence NM 031598.1 with an amplicon length of 94 bp. A β-actin primer and probe was used as a control, with the following sequences: Forward 5'GCCCTGGCTCCTAGCACC-3', Reverse 5'CCACCAATCCACACAGAGTACTTG-3', and Probe 5'TGAAGATCAAGTCATTGCTCCTCCTGAGC-3' with a FAM modification on the 5' end, having an amplicon length of 73 bp. Briefly, 5 µl cDNA was combined with Taqman Universal PCR Master Mix, along with a FAM-MGB probe and primers, for a final concentration of 25 µl. The reaction plate was incubated as follows: 50 ^{o}C for 2 min, 95 ^{o} C for 10 min, and 50 cycles of denature (95 ^{o}C for 15 sec) and anneal/extend (60 ^{o}C for 1 min). The C_T was determined and sPLA2-IIA transcript concentration was normalized against that of β-actin.

2.8. Statistical analysis

Data were analyzed by one-way ANOVA followed by Newman-Keuls multiple comparison test (V4.00, GraphPad Prism Software, Inc., San Diego, CA). Values of $p<0.05$ were accepted as significant.

3. RESULTS

3.1. Cytokine induction of sPLA2-IIA mRNA and protein expression in rat immortalized astrocytes (DITNC)

In the initial study, immortalized astrocytes (DITNC) were tested for induction of sPLA2-IIA mRNA expression by pro-inflammatory cytokines. The RT-PCR study indicated induction of sPLA2-IIA mRNA upon exposure to IL-1β and TNF-α for 18 h (Fig 1A). Exposure of astrocytes with interferon gamma (IFN-γ) alone did not cause the induction of sPLA2-IIA mRNA (data not shown).

Western blot analysis was used to estimate sPLA2-IIA concentrations in the cell lysates and in the culture medium. As shown in Fig 1B, exposure of astrocytes to TNF-α and IL-1β for 48 h induced visible sPLA2-IIA protein in both cell lysate and in the culture medium. Similar induction profile was observed in primary rat astrocytes (data not shown).

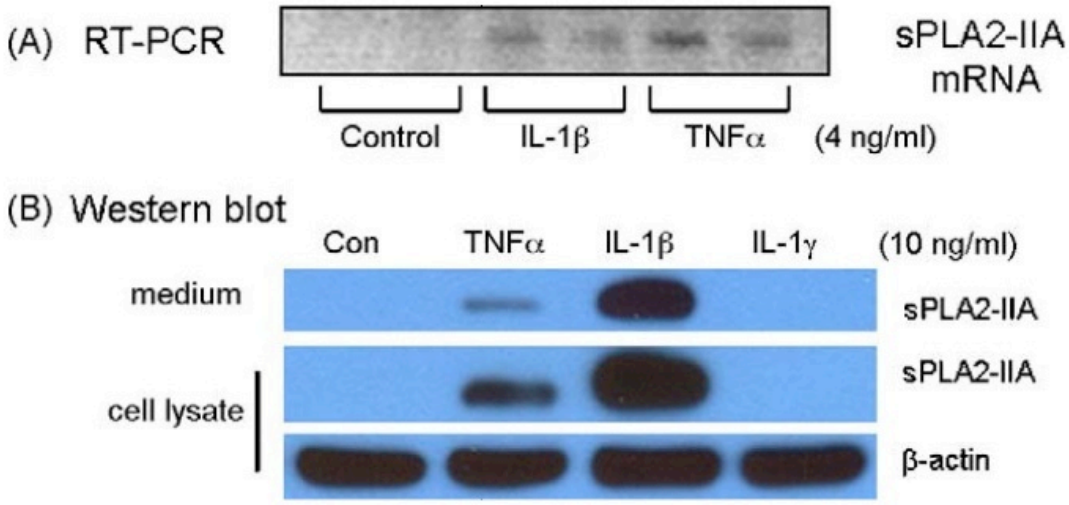

Figure 3.1 - (A) Induction of sPLA2-IIA mRNA in immortalized rat astrocytes (DITNC) by IL-1b and TNFa. Astrocytes were treated with 4 ng/ml with IL-1β or TNF-α for 18 h prior to measurement of sPLA2-IIA mRNA expression by RT-PCR. Results are representative of three independent experiments. (B) Induction of sPLA2-IIA protein in astrocytes. DITNC astrocytes were cultured in 60 mm dish and serum-starved for 4 h prior to treatment with TNF-α, IL-1β and IFN-γ (10 ng/ml) for 48 h. After treatment, culture medium was removed, cells washed with PBS and lysis buffer was added. Medium (40 ml) and cell lysate (15 mg protein) was used in Western blot as described in text. β-actin in cell lysate was used as control.

3.2. Involvement of protein kinases in the induction of sPLA2-IIA mRNA and protein by IL-1β

We tested the effects of several protein kinases, i.e., ERK1/2, p38 MAPK and PI-3 kinase, on induction of sPLA2-IIA by IL-1β. The MEK inhibitor, U0126, which inhibits MEK1/2, a direct activator of ERK1/2, was effective in inhibiting IL-1β-induced expression of sPLA2-IIA mRNA and protein (Fig 2). However, SB203580, an inhibitor for p38 MAPK, did not alter IL-1β induced sPLA2-IIA expression (data not shown). As shown in Figure 3, IL-1β induced sPLA2-IIA up-regulation in DITNC cells was significantly attenuated by LY294002, a known inhibitor of PI-3 kinase.

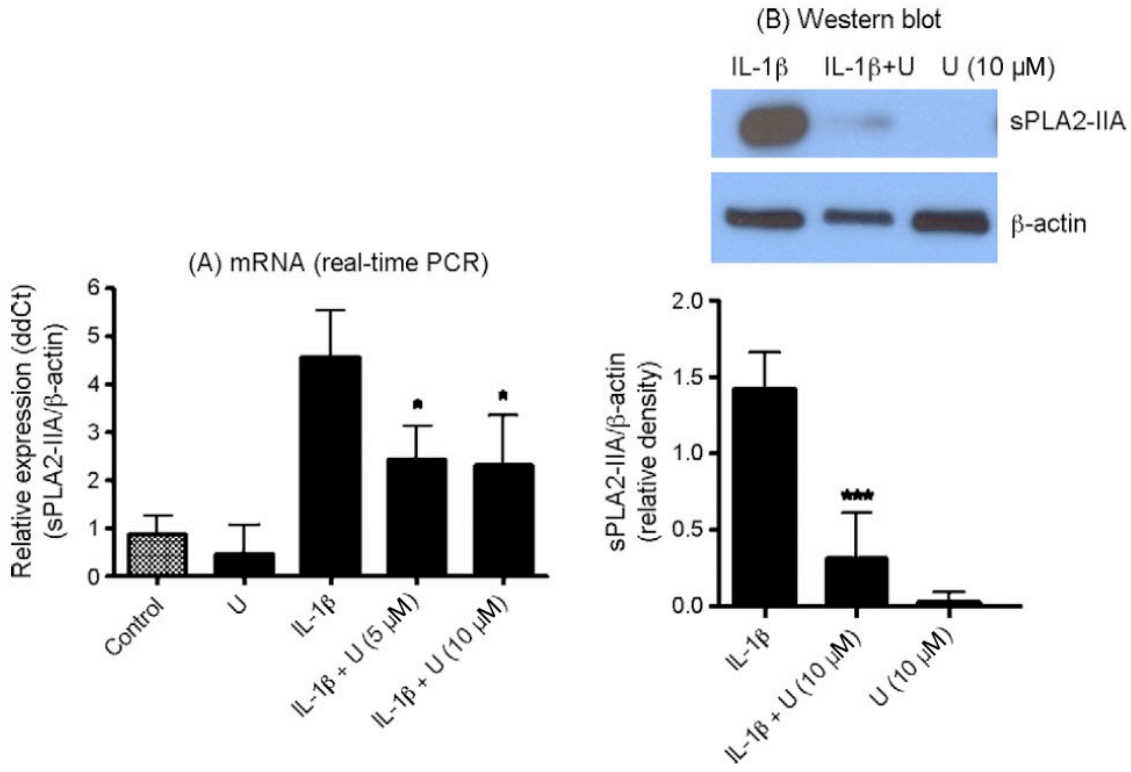

Figure 3.2 - U0126 (U), a MEK inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA and protein expressions in astrocytes. (A) For mRNA expression, astrocytes were treated with U0126 (10 mM) alone, IL-1β(4 ng/ml) alone and IL-1β with U0126 (5 and 10 mM) for 18 h. U0126 was added to astrocytes 1 h before treating with IL-1β. sPLA2-IIA expression was measured by real-time PCR relative to b-actin expression as described in text. Results are mean ± SD from three experiments. (B) For protein expression, astrocytes were treated with U0126 (10 mM), IL-1β (10 ng/ml), and IL-1β + U0126 for 48 h. U0126 was added 1 h prior to adding IL-1β. After incubation, cell lysates were taken for Western blot analysis as described in text. β-actin was used as control. Results are mean ± SD from three experiments. *p<0.05, **p<0.01, ***p<0.001 vs. IL-1b.

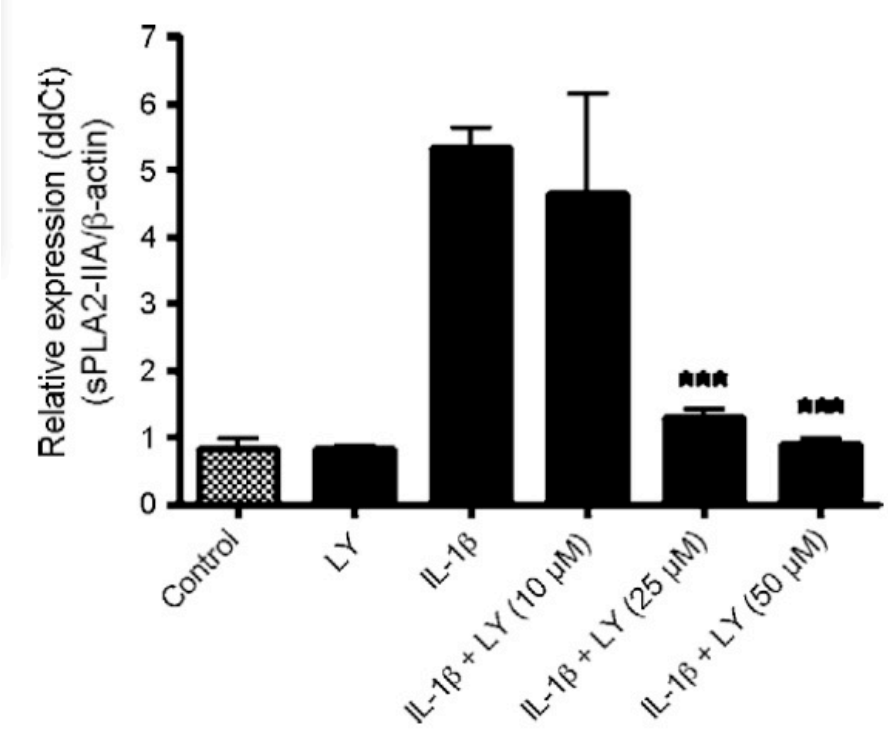

Figure 3.3 - LY294002, a PI-3 kinase inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with LY294002 (LY, 50 mM), IL-1b (4 ng/ml), and IL-1b with LY at 10, 25, and 50 mM for 18 h. LY was added to astrocytes 1 h prior to treatment with IL-1b. sPLA2-IIA mRNA expression was determined by real-time PCR with b-actin as control. Results are mean $\pm$ SD from three experiments. ***$p<0.001$ vs. IL-1b.

3.3. Inhibition of IL-1β-induced sPLA2-IIA mRNA expression by apocynin

We tested the possible involvement of NADPH oxidase in mediating IL-1β-induced sPLA2-IIA expression by treating astrocytes with apocynin, an inhibitor known to block the translocation of cytoplasmic subunits from docking with membrane subunits of NADPH oxidase (Stolk et al., 1994). As shown in Fig 4, apocynin at 500 mM

effectively inhibited IL-1β-induced PLA2-IIA mRNA expression in astrocytes. Apocynin as well as the kinase inhibitors showed little to no effect on cell viability as demonstrated by MTT test (data not shown).

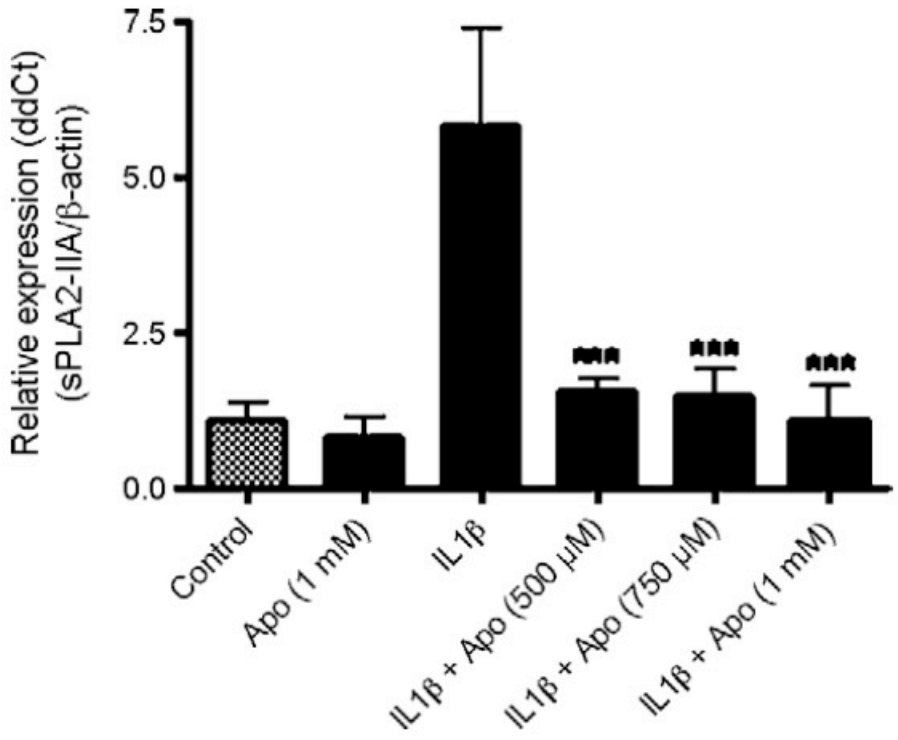

Figure 3.4 - Apocynin, an NADPH oxidase inhibitor, inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with apocynin (Apo, 1 mM), IL-1β (4 ng/ml), and IL-1β with apocynin at 500, 750 and 1000 mM for 18 h. Apocynin was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***p<0.001 vs. IL-1b.

3.4. Botanical polyphenols inhibited IL-1β-induced sPLA2-IIA mRNA through NADPH oxidase pathway

In this experiment, we tested the effects of resveratrol and EGCG on IL-1β-induced sPLA2-IIA in astrocytes. Our results show that pretreatment of astrocytes with

resveratrol resulted in an inhibition of IL-1β-induced expression of sPLA2-IIA mRNA (Fig 5). Similarly, pretreatment of astrocytes with EGCG also resulted in a dose-dependent inhibition of IL-1β-induced sPLA2-IIA mRNA expression (Fig 6).

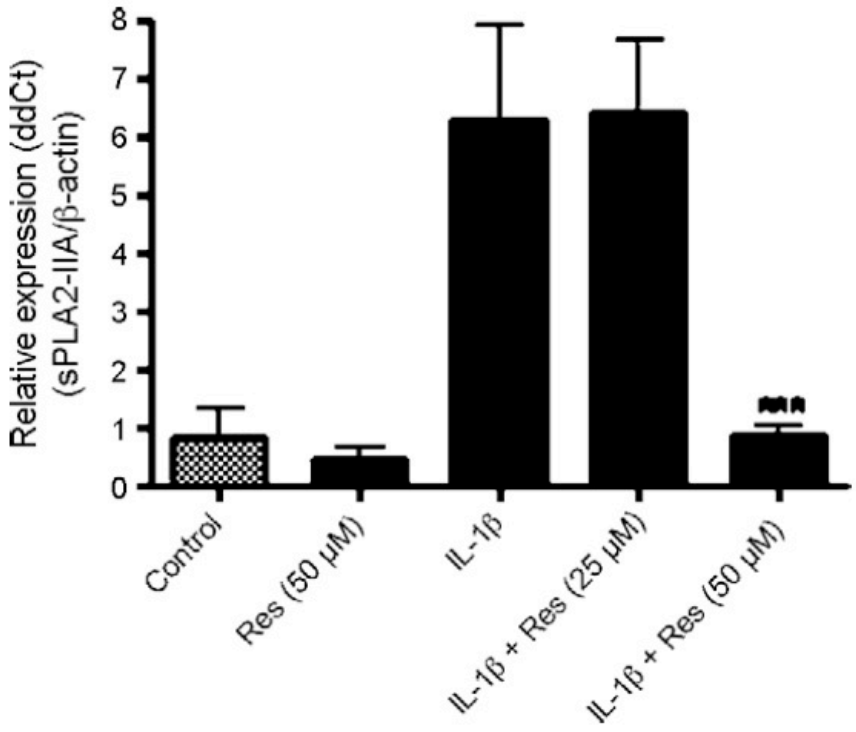

Figure 3.5 - Resveratrol (Res) inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with resveratrol (50 mM), IL-1β (4 ng/ml) and IL-1β with resveratrol at 25 or 50 mM for 18 h. Resveratrol was added to astrocytes 1 h prior to treatment with IL-1β. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***p<0.001 vs. IL-1b.

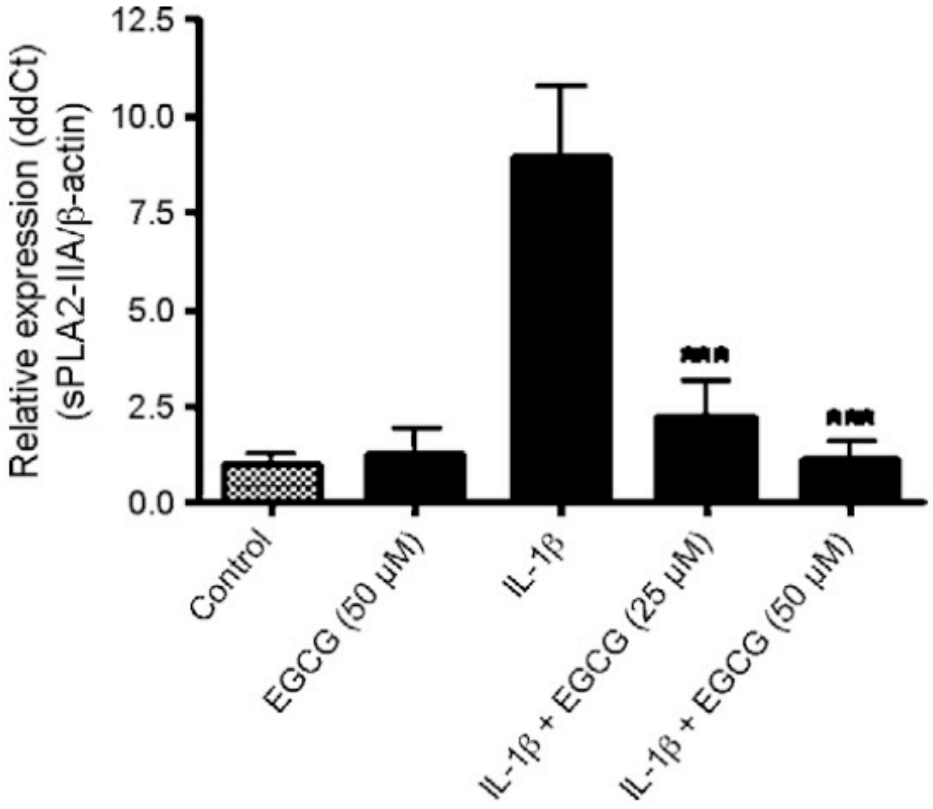

Figure 3.6 - Epigallocatechin gallate (EGCG) inhibited IL-1β-induced sPLA2-IIA mRNA expression in astrocytes. Astrocytes were treated with EGCG (50 mM), IL-1β (4 ng/ml) and IL-1β with EGCG at 25 or 50 mM for 18 h. EGCG was added to astrocytes 1 h prior to treatment with IL-1b. sPLA2-IIA mRNA expression was determined using real-time PCR with β-actin as control. Results are mean ± SD from three experiments. ***p<0.001 vs. IL-1b.

With both resveratrol and EGCG inhibiting sPLA2-IIA mRNA expression, we further tested whether these compounds inhibit the translocation of the soluble NADPH oxidase subunit p67 phox to membranes. After pretreatment with resveratrol or EGCG, astrocytes were exposed to IL-1β for 10 min and expression of p67 phox protein in the

membrane fraction was examined by Western blot analysis. As shown in Fig 7, IL-1β induced the translocation of p67 phox to the membrane fraction and resveratrol and EGCG each inhibited this translocation.

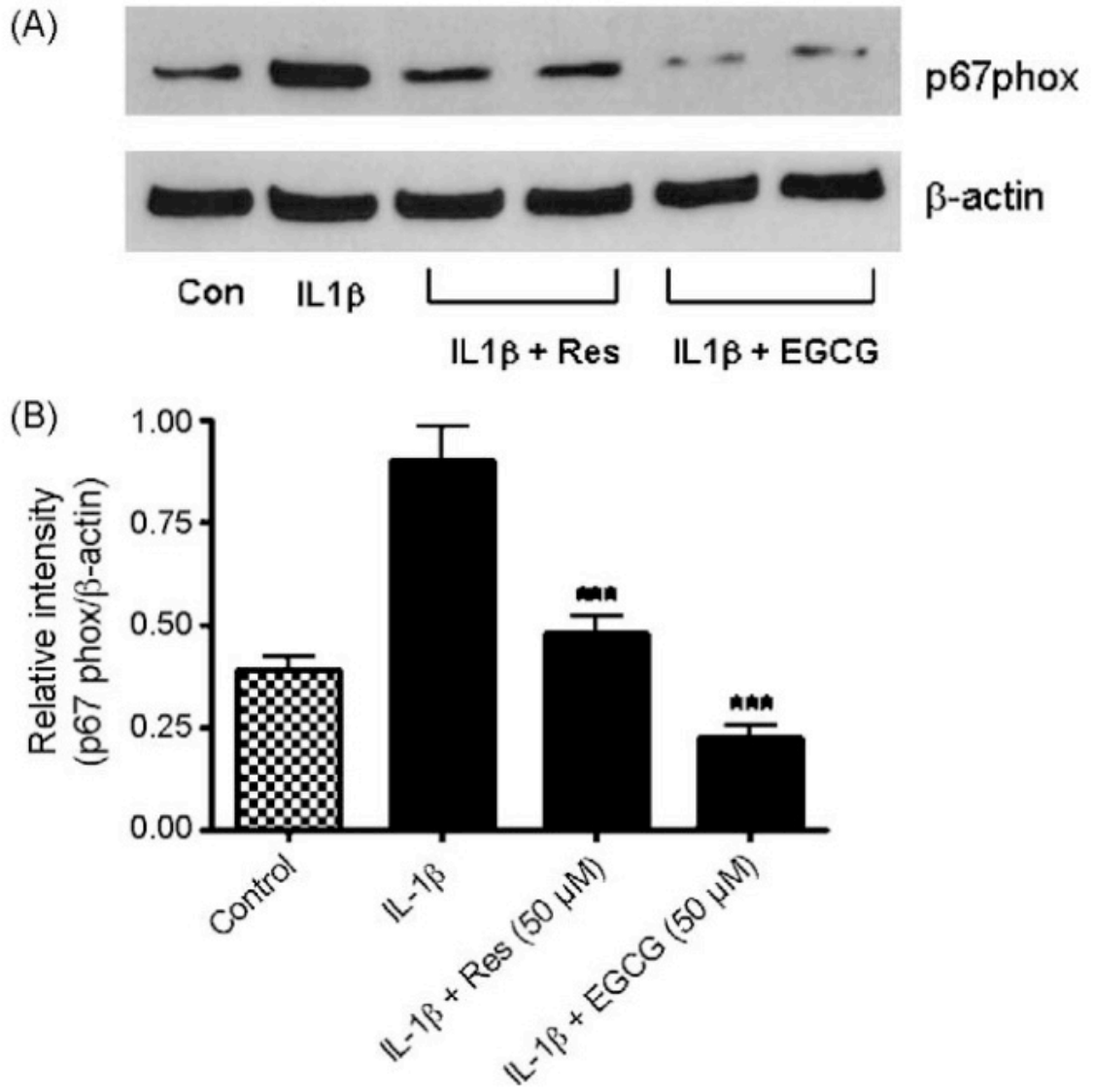

Figure 3.7 - Resveratrol and EGCG inhibited IL-1β-induced translocation of p67 phox to the membrane fraction. Astrocytes were treated with IL-1β (4 ng/ml) or IL-1β plus Res (50 mM) and EGCG (50 mM) for 10 min. Cells were disrupted and cell cytosol and membrane fractions were separated by centrifugation as described in Method section. (A) Western blot analysis of p67 phox concentration in the membrane fraction compared to β-actin as control. (B) Relative intensity of p67 phox compared to β-actin. Results are mean ± SD from three independent experiments. ***p<0.001 vs. IL-1b.

3.5. Apocynin and botanical polyphenols inhibited IL-1b-induced ERK1/2 phosphorylation

Since ERK1/2 has been shown to be activated following NADPH oxidase activation in astrocytes (Pawate et al., 2004), we investigated the effects of apocynin, resveratrol, and EGCG on the phosphorylation of ERK1/2 after stimulation of astrocytes with IL-1β. As shown in Fig 8, all three compounds inhibited IL-1β-induced increase in phospho-p42 ERK.

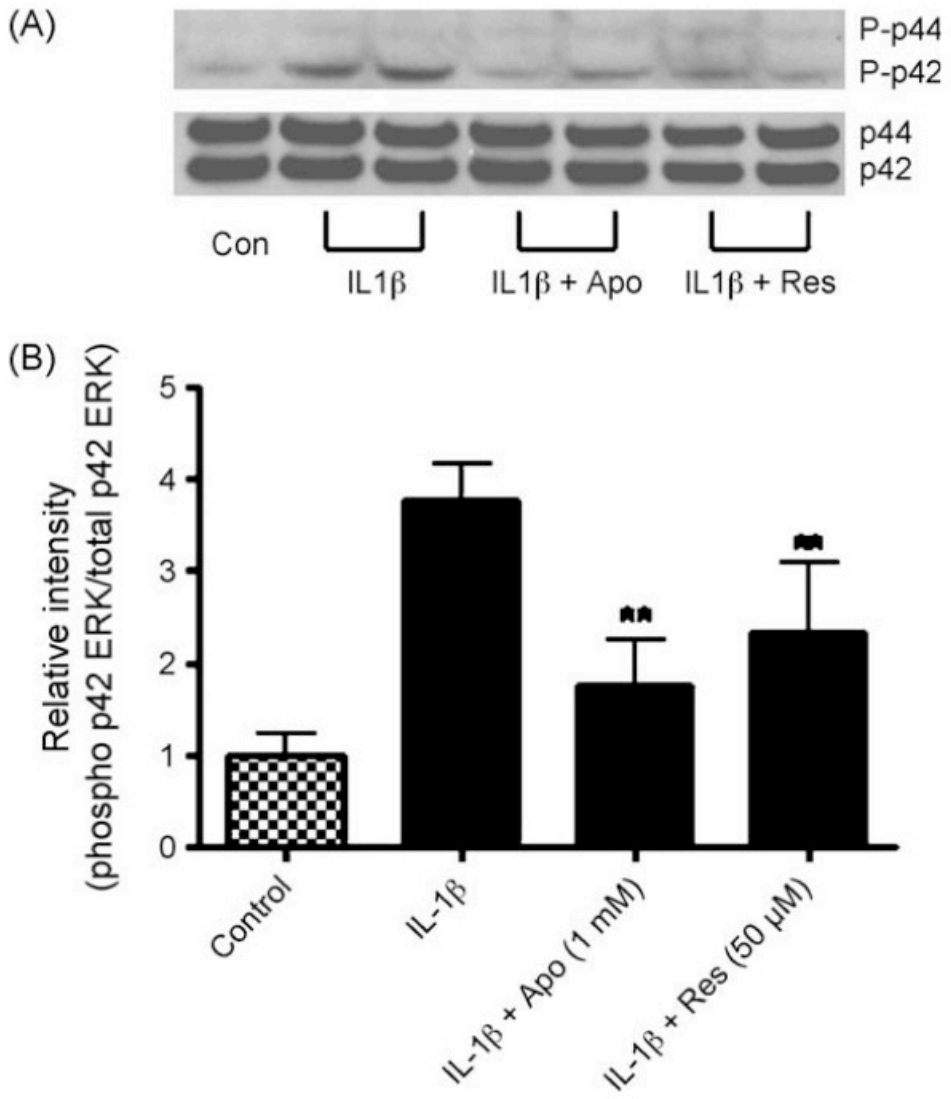

Figure 3.8 - Apocynin and resveratrol inhibited IL-1b-induced ERK1/2 phosphorylation in astrocytes. Astrocytes were treated with IL-1β (4 ng/ml), or IL-1β plus apocynin (1 mM) and Res (50 mM) for 10 min. (A) Cell lysate was used for Western blot analysis for phospho-ERK1/2 and total ERK1/2. (B) Relative intensities of phospho-p42 ERK against total p42 ERK. Results are mean ± SD from three independent experiments. **$p<0.01$ vs. IL-1β.

4. DISCUSSION

The importance of sPLA2-IIA in neurodegenerative diseases, especially in association with inflammatory processes has started to emerge (Adibhatla and Hatcher, 2007; Sun et al., 2007). Studies linking this enzyme with transgenic mouse models have been hampered due to a missense mutation in the sPLA2-IIA gene in a number of mouse strains (Kennedy et al., 1995). However, studies with rat brain demonstrated the inflammatory properties of this enzyme and its upregulation in astrocytes by pro-inflammatory cytokines including IL-1β and TNF-α (Oka and Arita, 1991; Tong et al., 1999; Rosenberger et al., 2004; Moses et al., 2006). In the present study, our Western blot analysis demonstrates for the first time that cytokine-induced sPLA2-IIA protein is secreted into the culture medium. This result is in agreement with our previous study showing active PLA2 activity in the culture media using radioactive phospholipids as substrate (Xu et al., 2003).

Although a number of studies have demonstrated the transcriptional induction of sPLA2-IIA through the NF-κB pathway (Andreani et al., 2000; Lappas et al., 2004), less

is known about the roles of other signaling molecules in mediating the induction. In this study, we used real-time PCR to measure sPLA2-IIA mRNA expression in astrocytes, and demonstrated the involvement of ERK1/2 and PI-3 kinase (but not p38 MAPK) in sPLA2-IIA expression by IL-1b. In the CNS, ERK1/2 are important signaling molecules that integrate extracellular signals (Sweatt, 2001; Chu et al., 2004). Furthermore, ERK1/2 activation is implicated in a large number of intracellular factors, including Ca^{2+}, PKC, nitric oxide, and PI-3 kinase (Andersen et al., 2003). In our recent studies with cortical neurons, ERK1/2 phosphorylation was stimulated by ROS produced by NADPH oxidase and in turn this signaling pathway led to activation of cPLA2 (Shelat et al., 2008). Other studies have also demonstrated that NADPH oxidase-generated ROS can activate the ERK1/2 pathway and vice versa, ERK1/2 activation can stimulate the phosphorylation of cytosolic subunits of NAPDH oxidase leading to an increased activity of the enzyme (Pawate et al., 2004; Miller et al., 2007; Yang et al., 2007a). In microglial cells, LPS-induced activation of PI-3 kinase and p38 MAPK (but not ERK1/2) pathways is dependent on ROS production by NADPH oxidase (Sun et al., 2008b). These kinases are important in glial redox signaling and their inhibition can lead to the reduced production of inflammatory proteins (Bhat et al., 1998; Saha and Pahan, 2006; Yang et al., 2007b). Our results are in agreement with these findings. Interestingly, PKC activation of PI-3 kinase (Frey et al., 2006) can modulate sPLA2-IIA expression in IL-1β treated mesangial cells (Scholz et al., 1999). Apparently, depending on the cell types, cytokines may stimulate different kinase pathways and regulate the transcriptional events leading to sPLA2-IIA expression.

Although the mechanism is not well understood, ROS have been shown to play a role as second messengers in NF-κB activation (Schreck et al., 1992; Baeuerle and Henkel, 1994; Flohe et al., 1997). In this study, we provided evidence that NADPH oxidase-derived ROS is necessary for IL-1β induction of sPLA2-IIA. Our results are in accordance with other findings which demonstrated the involvement of NADPH oxidase in neuroinflammatory processes, especially glial activation (Brown, 2007). NADPH oxidase is considered an important non-mitochondrial source of oxidative stress in the brain and has been implicated in a number of neurodegenerative diseases including Alzheimer's, Parkinson's, HIV dementia, ischemic stroke, and multiple sclerosis (Bedard and Krause, 2007). The involvement of NADPH oxidase in IL-1β-mediated induction of sPLA2-IIA was demonstrated by inhibition with apocynin, a phenolic compound extracted from *Picrorhiza kurroa*, a creeping plant native to the mountains of India, Nepal, Tibet and Pakistan (Wang et al., 2008). Apocynin has been shown to block the activity of NADPH oxidase by interfering with the assembly of the cytosolic subunits with the membrane components (Stolk et al., 1994). The ability for apocynin to inhibit translocation of p67 phox to membranes is also indication for the involvement of NADPH oxidase in this process.

Due to their antioxidant properties, there is considerable interest to examine whether polyphenolic compounds including resveratrol from grapes and EGCG from green tea may offer protective effects and ameliorate progression of neurodegenerative diseases (Joseph et al., 2000; Zaveri, 2006; Cucciolla et al., 2007; Mancuso et al., 2007; Son et al., 2008). Our earlier studies have demonstrated the abilities of resveratrol and also apocynin to protect against neuronal injury and glial activation due to cerebral

ischemia-reperfusion (Wang et al., 2002; Wang et al., 2006). Other studies also demonstrated the ability of antioxidant botanical compounds to suppress the NF-kB pathway (Packer, 1998; Lavrovsky et al., 2000; Cindrova-Davies et al., 2007). Indeed, recent findings have indicated that the neuroprotective actions of these compounds arise from their modulation of important cellular signaling pathways (Mandel et al., 2004). In the current study, apocynin, resveratrol and EGCG were shown to inhibit IL-1b-induced sPLA2-IIA mRNA expression in astrocytes. The fact that these compounds could inhibit p67 phox translocation and IL-Iβ induced ERK activation further supports the notion that these antioxidants may act by targeting the NADPH oxidase pathway. Our results are in agreement with recent study indicating that both resveratrol and EGCG could inhibit NADPH oxidase activity in endothelial cells (Steffen et al., 2008).

In conclusion, this study provides evidence that protein kinases and oxidative pathways, most likely the NADPH oxidase pathway, promote the expression of sPLA2-IIA mRNA in astrocytes. This study further provides support for the down regulation of this inflammatory protein by inhibitors of NADPH oxidase and botanical antioxidants.

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REFERENCES

Aarsman AJ, Neys FW, van der Helm HA, Kuypers FA, van den Bosch H (2000) Sera of patients suffering from inflammatory diseases contain group IIA but not group V phospholipase A(2). Biochim Biophys Acta 1502:257-263.

Abramov AY, Canevari L, Duchen MR (2004) Beta-amyloid peptides induce mitochondrial dysfunction and oxidative stress in astrocytes and death of neurons through activation of NADPH oxidase. J Neurosci 24:565-575.

Abramov AY, Jacobson J, Wientjes F, Hothersall J, Canevari L, Duchen MR (2005) Expression and modulation of an NADPH oxidase in mammalian astrocytes. J Neurosci 25:9176-9184.

Adibhatla RM, Hatcher JF (2006) Phospholipase A2, reactive oxygen species, and lipid peroxidation in cerebral ischemia. Free Radical Biol Med 40:376-387.

Adibhatla RM, Hatcher JF (2007) Secretory phospholipase A2 IIA is up-regulated by TNF-alpha and IL-1alpha/beta after transient focal cerebral ischemia in rat. Brain research 1134:199-205.

Adibhatla RM, Hatcher JF, Larsen EC, Chen X, Sun D, Tsao FH (2006) CDP-choline significantly restores phosphatidylcholine levels by differentially affecting phospholipase A2 and CTP: phosphocholine cytidylyltransferase after stroke. The Journal of biological chemistry 281:6718-6725.

Akama KT, Albanese C, Pestell RG, Van Eldik LJ (1998) Amyloid beta-peptide stimulates nitric oxide production in astrocytes through an NFkappaB-dependent mechanism. Proc Natl Acad Sci U S A 95:5795-5800.

Akiba S, Hatazawa R, Ono K, Kitatani K, Hayama M, Sato T (2001) Secretory phospholipase A2 mediates cooperative prostaglandin generation by growth factor and cytokine independently of preceding cytosolic phospholipase A2 expression in rat gastric epithelial cells. The Journal of biological chemistry 276:21854-21862.

Akiyama H et al. (2000) Inflammation and Alzheimer's disease. Neurobiol Aging 21:383-421.

Andersen JM, Myhre O, Fonnum F (2003) Discussion of the role of the extracellular signal-regulated kinase-phospholipase A2 pathway in production of reactive oxygen species in Alzheimer's disease. Neurochemical research 28:319-326.

Andreani M, Olivier JL, Berenbaum F, Raymondjean M, Bereziat G (2000) Transcriptional regulation of inflammatory secreted phospholipases A(2). Biochim Biophys Acta 1488:149-158.

Atsumi G, Murakami M, Tajima M, Shimbara S, Hara N, Kudo I (1997) The perturbed membrane of cells undergoing apoptosis is susceptible to type II secretory phospholipase A2 to liberate arachidonic acid. Biochim Biophys Acta 1349:43-54.

Baeuerle PA, Henkel T (1994) Function and activation of NF-kappa B in the immune system. Annual review of immunology 12:141-179.

Balboa MA, Varela-Nieto I, Killermann Lucas K, Dennis EA (2002) Expression and function of phospholipase A(2) in brain. FEBS Lett 531:12-17.

Balboa MA, Balsinde J, Winstead MV, Tischfield JA, Dennis EA (1996) Novel group V phospholipase A2 involved in arachidonic acid mobilization in murine P388D1 macrophages. The Journal of biological chemistry 271:32381-32384.

Balestrieri B, Hsu VW, Gilbert H, Leslie CC, Han WK, Bonventre JV, Arm JP (2006) Group V secretory phospholipase A2 translocates to the phagosome after zymosan stimulation of mouse peritoneal macrophages and regulates phagocytosis. The Journal of biological chemistry 281:6691-6698.

Barbieri SS, Cavalca V, Eligini S, Brambilla M, Caiani A, Tremoli E, Colli S (2004) Apocynin prevents cyclooxygenase 2 expression in human monocytes through NADPH oxidase and glutathione redox-dependent mechanisms. Free radical biology & medicine 37:156-165.

Bastianetto S, Yao ZX, Papadopoulos V, Quirion R (2006) Neuroprotective effects of green and black teas and their catechin gallate esters against beta-amyloid-induced toxicity. The European journal of neuroscience 23:55-64.

Bazan NG (2003) Synaptic lipid signaling: significance of polyunsaturated fatty acids and platelet-activating factor. J Lipid Res 44:2221-2233.

Bazan NG (2005) Synaptic signaling by lipids in the life and death of neurons. Mol Neurobiol 31:219-230.

Bedard K, Krause KH (2007) The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiological reviews 87:245-313.

Bezzine S, Koduri RS, Valentin E, Murakami M, Kudo I, Ghomashchi F, Sadilek M, Lambeau G, Gelb MH (2000) Exogenously added human group X secreted phospholipase A(2) but not the group IB, IIA, and V enzymes efficiently release arachidonic acid from adherent mammalian cells. The Journal of biological chemistry 275:3179-3191.

Bhat NR, Zhang P, Lee JC, Hogan EL (1998) Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-alpha gene expression in endotoxin-stimulated primary glial cultures. J Neurosci 18:1633-1641.

Bingham CO, 3rd, Fijneman RJ, Friend DS, Goddeau RP, Rogers RA, Austen KF, Arm JP (1999) Low molecular weight group IIA and group V phospholipase A(2) enzymes have different intracellular locations in mouse bone marrow-derived mast cells. The Journal of biological chemistry 274:31476-31484.

Boilard E, Bourgoin SG, Bernatchez C, Poubelle PE, Surette ME (2003) Interaction of low molecular weight group IIA phospholipase A2 with apoptotic human T cells: role of heparan sulfate proteoglycans. Faseb J 17:1068-1080.

Boilard E, Rouault M, Surrel F, Le Calvez C, Bezzine S, Singer A, Gelb MH, Lambeau G (2006) Secreted phospholipase A2 inhibitors are also potent blockers of binding to the M-type receptor. Biochemistry 45:13203-13218.

Bostrom MA, Boyanovsky BB, Jordan CT, Wadsworth MP, Taatjes DJ, de Beer FC, Webb NR (2007) Group v secretory phospholipase A2 promotes atherosclerosis: evidence from genetically altered mice. Arteriosclerosis, thrombosis, and vascular biology 27:600-606.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.

Brown GC (2007) Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. Biochem Soc Trans 35:1119-1121.

Burke JE, Dennis EA (2008a) Phospholipase A2 structure/function, mechanism and signaling. Journal of lipid research.

Burke JE, Dennis EA (2008b) Phospholipase A(2) Biochemistry. Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy.

Burke JE, Dennis EA (2009a) Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res 50 Suppl:S237-242.

Burke JE, Dennis EA (2009b) Phospholipase A2 biochemistry. Cardiovasc Drugs Ther 23:49-59.

Butterfield DA (1997) beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. Chem Res Toxicol 10:495-506.

Butterfield DA, Lauderback CM (2002) Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. Free Radic Biol Med 32:1050-1060.

Cacquevel M, Lebeurrier N, Cheenne S, Vivien D (2004) Cytokines in neuroinflammation and Alzheimer's disease. Curr Drug Targets 5:529-534.

Carey MC, Small DM, Bliss CM (1983) Lipid digestion and absorption. Annu Rev Physiol 45:651-677.

Cerf E, Sarroukh R, Tamamizu-Kato S, Breydo L, Derclaye S, Dufrene YF, Narayanaswami V, Goormaghtigh E, Ruysschaert JM, Raussens V (2009) Antiparallel beta-sheet: a signature structure of the oligomeric amyloid beta-peptide. The Biochemical journal 421:415-423.

Chen C, Yu R, Owuor ED, Kong AN (2000) Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death. Arch Pharm Res 23:605-612.

Chen J, Engle SJ, Seilhamer JJ, Tischfield JA (1994a) Cloning and recombinant expression of a novel human low molecular weight Ca(2+)-dependent phospholipase A2. The Journal of biological chemistry 269:2365-2368.

Chen J, Engle SJ, Seilhamer JJ, Tischfield JA (1994b) Cloning, expression and partial characterization of a novel rat phospholipase A2. Biochimica et biophysica acta 1215:115-120.

Chen J, Shao C, Lazar V, Srivastava CH, Lee WH, Tischfield JA (1997) Localization of group IIc low molecular weight phospholipase A2 mRNA to meiotic cells in the mouse. J Cell Biochem 64:369-375.

Chow SE, Hshu YC, Wang JS, Chen JK (2007) Resveratrol attenuates oxLDL-stimulated NADPH oxidase activity and protects endothelial cells from oxidative functional damages. J Appl Physiol 102:1520-1527.

Chu CT, Levinthal DJ, Kulich SM, Chalovich EM, DeFranco DB (2004) Oxidative neuronal injury. The dark side of ERK1/2. Eur J Biochem 271:2060-2066.

Chung JH, Han JH, Hwang EJ, Seo JY, Cho KH, Kim KH, Youn JI, Eun HC (2003) Dual mechanisms of green tea extract (EGCG)-induced cell survival in human epidermal keratinocytes. Faseb J 17:1913-1915.

Cindrova-Davies T, Spasic-Boskovic O, Jauniaux E, Charnock-Jones DS, Burton GJ (2007) Nuclear factor-kappa B, p38, and stress-activated protein kinase mitogen-activated protein kinase signaling pathways regulate proinflammatory cytokines and apoptosis in human placental explants in response to oxidative stress: effects of antioxidant vitamins. The American journal of pathology 170:1511-1520.

Colangelo V, Schurr J, Ball MJ, Pelaez RP, Bazan NG, Lukiw WJ (2002) Gene expression profiling of 12633 genes in Alzheimer hippocampal CA1: transcription and neurotrophic factor down-regulation and up-regulation of apoptotic and pro-inflammatory signaling. J Neurosci Res 70:462-473.

Craft JM, Watterson DM, Van Eldik LJ (2006) Human amyloid beta-induced neuroinflammation is an early event in neurodegeneration. Glia 53:484-490.

Cucciolla V, Borriello A, Oliva A, Galletti P, Zappia V, Della Ragione F (2007) Resveratrol: from basic science to the clinic. Cell cycle (Georgetown, Tex 6:2495-2510.

Cunningham TJ, Souayah N, Jameson B, Mitchell J, Yao L (2004) Systemic treatment of cerebral cortex lesions in rats with a new secreted phospholipase A2 inhibitor. J Neurotrauma 21:1683-1691.

Cupillard L, Koumanov K, Mattei MG, Lazdunski M, Lambeau G (1997) Cloning, chromosomal mapping, and expression of a novel human secretory phospholipase A2. The Journal of biological chemistry 272:15745-15752.

Dahlgren KN, Manelli AM, Stine WB, Jr., Baker LK, Krafft GA, LaDu MJ (2002) Oligomeric and fibrillar species of amyloid-beta peptides differentially affect neuronal viability. The Journal of biological chemistry 277:32046-32053.

Dennis EA (1994) Diversity of group types, regulation, and function of phospholipase A2. The Journal of biological chemistry 269:13057-13060.

Dworakowski R, Anilkumar N, Zhang M, Shah AM (2006) Redox signalling involving NADPH oxidase-derived reactive oxygen species. Biochemical Society transactions 34:960-964.

Eikelenboom P, Veerhuis R (1996) The role of complement and activated microglia in the pathogenesis of Alzheimer's disease. Neurobiol Aging 17:673-680.

El Benna J, Han J, Park JW, Schmid E, Ulevitch RJ, Babior BM (1996) Activation of p38 in stimulated human neutrophils: phosphorylation of the oxidase component p47phox by p38 and ERK but not by JNK. Archives of biochemistry and biophysics 334:395-400.

Farbman AI, Brann JH, Rozenblat A, Rochlin MW, Weiler E, Bhattacharyya M (2004) Developmental expression of neurotrophin receptor genes in rat geniculate ganglion neurons. J Neurocytol 33:331-343.

Farooqui AA, Horrocks LA (2006) Phospholipase A2-generated lipid mediators in the brain: the good, the bad, and the ugly. Neuroscientist 12:245-260.

Farooqui AA, Yang HC, Horrocks L (1997a) Involvement of phospholipase A2 in neurodegeneration. Neurochem Int 30:517-522.

Farooqui AA, Yang HC, Rosenberger TA, Horrocks LA (1997b) Phospholipase A2 and its role in brain tissue. J Neurochem 69:889-901.

Farooqui AA, Litsky ML, Farooqui T, Horrocks LA (1999) Inhibitors of intracellular phospholipase A2 activity: their neurochemical effects and therapeutical importance for neurological disorders. Brain Res Bull 49:139-153.

Flohe L, Brigelius-Flohe R, Saliou C, Traber MG, Packer L (1997) Redox regulation of NF-kappa B activation. Free radical biology & medicine 22:1115-1126.

Floyd RA (1999) Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med 222:236-245.

Floyd RA, Carney JM (1992) Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Ann Neurol 32 Suppl:S22-27.

Fourcade O, Le Balle F, Fauvel J, Simon MF, Chap H (1998) Regulation of secretory type-II phospholipase A2 and of lysophosphatidic acid synthesis. Adv Enzyme Regul 38:99-107.

Frey RS, Gao X, Javaid K, Siddiqui SS, Rahman A, Malik AB (2006) Phosphatidylinositol 3-kinase gamma signaling through protein kinase Czeta induces NADPH oxidase-mediated oxidant generation and NF-kappaB activation in endothelial cells. The Journal of biological chemistry 281:16128-16138.

Fuentes L, Hernandez M, Fernandez-Aviles FJ, Crespo MS, Nieto ML (2002) Cooperation between secretory phospholipase A2 and TNF-receptor superfamily signaling: implications for the inflammatory response in atherogenesis. Circ Res 91:681-688.

Fujioka D, Saito Y, Kobayashi T, Yano T, Tezuka H, Ishimoto Y, Suzuki N, Yokota Y, Nakamura T, Obata JE, Kanazawa M, Kawabata K, Hanasaki K, Kugiyama K (2008) Reduction in myocardial ischemia/reperfusion injury in group X secretory phospholipase A2-deficient mice. Circulation 117:2977-2985.

Gella A, Durany N (2009) Oxidative stress in Alzheimer disease. Cell Adh Migr 3.

Ghosh M, Tucker DE, Burchett SA, Leslie CC (2006) Properties of the Group IV phospholipase A2 family. Prog Lipid Res 45:487-510.

Griffin WS (2006) Inflammation and neurodegenerative diseases. Am J Clin Nutr 83:470S-474S.

Griffin WS, Mrak RE (2002) Interleukin-1 in the genesis and progression of and risk for development of neuronal degeneration in Alzheimer's disease. J Leukoc Biol 72:233-238.

Halliday G, Robinson SR, Shepherd C, Kril J (2000) Alzheimer's disease and inflammation: a review of cellular and therapeutic mechanisms. Clinical and experimental pharmacology & physiology 27:1-8.

Han WK, Sapirstein A, Hung CC, Alessandrini A, Bonventre JV (2003) Cross-talk between cytosolic phospholipase A2 alpha (cPLA2 alpha) and secretory phospholipase A2 (sPLA2) in hydrogen peroxide-induced arachidonic acid release in murine mesangial cells: sPLA2 regulates cPLA2 alpha activity that is responsible for arachidonic acid release. The Journal of biological chemistry 278:24153-24163.

Han YS, Zheng WH, Bastianetto S, Chabot JG, Quirion R (2004) Neuroprotective effects of resveratrol against beta-amyloid-induced neurotoxicity in rat hippocampal neurons: involvement of protein kinase C. British journal of pharmacology 141:997-1005.

Hanasaki K, Arita H (2002) Phospholipase A2 receptor: a regulator of biological functions of secretory phospholipase A2. Prostaglandins Other Lipid Mediat 68-69:71-82.

Hanasaki K, Ono T, Saiga A, Morioka Y, Ikeda M, Kawamoto K, Higashino K, Nakano K, Yamada K, Ishizaki J, Arita H (1999) Purified group X secretory phospholipase A(2) induced prominent release of arachidonic acid from human myeloid leukemia cells. The Journal of biological chemistry 274:34203-34211.

Haroutunian V, Perl DP, Purohit DP, Marin D, Khan K, Lantz M, Davis KL, Mohs RC (1998) Regional distribution of neuritic plaques in the nondemented elderly and subjects with very mild Alzheimer disease. Arch Neurol 55:1185-1191.

Hashmi N, Lee S, Cummings MP (2004) Abstracting Workflows: Unifying Bioinformatics Task Conceptualization and Specification Through Semantic Web Services. In, pp 1-7. Cambridge, Massachusetts USA.

Henderson WR, Jr., Chi EY, Bollinger JG, Tien YT, Ye X, Castelli L, Rubtsov YP, Singer AG, Chiang GK, Nevalainen T, Rudensky AY, Gelb MH (2007) Importance of group X-secreted phospholipase A2 in allergen-induced airway inflammation and remodeling in a mouse asthma model. The Journal of experimental medicine 204:865-877.

Hirabayashi T, Murayama T, Shimizu T (2004) Regulatory mechanism and physiological role of cytosolic phospholipase A2. Biol Pharm Bull 27:1168-1173.

Ho IC, Arm JP, Bingham CO, 3rd, Choi A, Austen KF, Glimcher LH (2001) A novel group of phospholipase A2s preferentially expressed in type 2 helper T cells. The Journal of biological chemistry 276:18321-18326.

Hoyal CR, Gutierrez A, Young BM, Catz SD, Lin JH, Tsichlis PN, Babior BM (2003) Modulation of p47PHOX activity by site-specific phosphorylation: Akt-dependent activation of the NADPH oxidase. Proceedings of the National Academy of Sciences of the United States of America 100:5130-5135.

Hu J, Akama KT, Krafft GA, Chromy BA, Van Eldik LJ (1998) Amyloid-beta peptide activates cultured astrocytes: morphological alterations, cytokine induction and nitric oxide release. Brain Res 785:195-206.

Hung LM, Su MJ, Chu WK, Chiao CW, Chan WF, Chen JK (2002) The protective effect of resveratrols on ischaemia-reperfusion injuries of rat hearts is correlated with antioxidant efficacy. British journal of pharmacology 135:1627-1633.

Hurt-Camejo E, Camejo G, Peilot H, Oorni K, Kovanen P (2001) Phospholipase A(2) in vascular disease. Circ Res 89:298-304.

Ibeas E, Fuentes L, Martin R, Hernandez M, Nieto ML (2009) Secreted phospholipase A2 type IIA as a mediator connecting innate and adaptive immunity: new role in atherosclerosis. Cardiovascular research 81:54-63.

Ikeno Y, Konno N, Cheon SH, Bolchi A, Ottonello S, Kitamoto K, Arioka M (2005) Secretory phospholipases A2 induce neurite outgrowth in PC12 cells through lysophosphatidylcholine generation and activation of G2A receptor. J Biol Chem 280:28044-28052.

Ishige K, Schubert D, Sagara Y (2001) Flavonoids protect neuronal cells from oxidative stress by three distinct mechanisms. Free radical biology & medicine 30:433-446.

Ishizaki J, Suzuki N, Higashino K, Yokota Y, Ono T, Kawamoto K, Fujii N, Arita H, Hanasaki K (1999) Cloning and characterization of novel mouse and human secretory phospholipase A(2)s. The Journal of biological chemistry 274:24973-24979.

Jamal OS, Conaghan PG, Cunningham AM, Brooks PM, Munro VF, Scott KF (1998) Increased expression of human type IIa secretory phospholipase A2 antigen in arthritic synovium. Ann Rheum Dis 57:550-558.

Jaulmes A, Janvier B, Andreani M, Raymondjean M (2005) Autocrine and paracrine transcriptional regulation of type IIA secretory phospholipase A2 gene in vascular smooth muscle cells. Arteriosclerosis, thrombosis, and vascular biology 25:1161-1167.

Jefferies WA, Food MR, Gabathuler R, Rothenberger S, Yamada T, Yasuhara O, McGeer PL (1996) Reactive microglia specifically associated with amyloid plaques in Alzheimer's disease brain tissue express melanotransferrin. Brain Res 712:122-126.

Jensen MD, Sheng W, Simonyi A, Johnson GS, Sun AY, Sun GY (2009) Involvement of oxidative pathways in cytokine-induced secretory phospholipase A2-IIA in astrocytes. Neurochem Int.

Jiang J, Neubauer BL, Graff JR, Chedid M, Thomas JE, Roehm NW, Zhang S, Eckert GJ, Koch MO, Eble JN, Cheng L (2002) Expression of group IIA secretory phospholipase A2 is elevated in prostatic intraepithelial neoplasia and adenocarcinoma. The American journal of pathology 160:667-671.

Joachim CL, Morris JH, Selkoe DJ (1989) Diffuse senile plaques occur commonly in the cerebellum in Alzheimer's disease. Am J Pathol 135:309-319.

Joseph JA, Denisova NA, Bielinski D, Fisher DR, Shukitt-Hale B (2000) Oxidative stress protection and vulnerability in aging: putative nutritional implications for intervention. Mechanisms of ageing and development 116:141-153.

Kashiwagi M, Friess H, Uhl W, Berberat P, Abou-Shady M, Martignoni M, Anghelacopoulos SE, Zimmermann A, Buchler MW (1999) Group II and IV phospholipase A(2) are produced in human pancreatic cancer cells and influence prognosis. Gut 45:605-612.

Kennedy BP, Payette P, Mudgett J, Vadas P, Pruzanski W, Kwan M, Tang C, Rancourt DE, Cromlish WA (1995) A natural disruption of the secretory group II phospholipase A2 gene in inbred mouse strains. J Biol Chem 270:22378-22385.

Kimura-Matsumoto M, Ishikawa Y, Komiyama K, Tsuruta T, Murakami M, Masuda S, Akasaka Y, Ito K, Ishiguro S, Morita H, Sato S, Ishii T (2008) Expression of secretory phospholipase A2s in human atherosclerosis development. Atherosclerosis 196:81-91.

Kolko M, de Turco EB, Diemer NH, Bazan NG (2002) Secretory phospholipase A2-mediated neuronal cell death involves glutamate ionotropic receptors. Neuroreport 13:1963-1966.

Kolko M, Christoffersen NR, Varoqui H, Bazan NG (2005) Expression and induction of secretory phospholipase A2 group IB in brain. Cellular and molecular neurobiology 25:1107-1122.

Kolko M, Christoffersen NR, Barreiro SG, Miller ML, Pizza AJ, Bazan NG (2006) Characterization and location of secretory phospholipase A2 groups IIE, V, and X in the rat brain. J Neurosci Res.

Krijnen PA, Meischl C, Nijmeijer R, Visser CA, Hack CE, Niessen HW (2006) Inhibition of sPLA2-IIA, C-reactive protein or complement: new therapy for patients with acute myocardial infarction? Cardiovascular & hematological disorders drug targets 6:113-123.

Kumar A, Sekharudu C, Ramakrishnan B, Dupureur CM, Zhu H, Tsai MD, Sundaralingam M (1994) Structure and function of the catalytic site mutant Asp 99 Asn of phospholipase A2: absence of the conserved structural water. Protein Sci 3:2082-2088.

Lafeber FP, Beukelman CJ, van den Worm E, van Roy JL, Vianen ME, van Roon JA, van Dijk H, Bijlsma JW (1999) Apocynin, a plant-derived, cartilage-saving drug, might be useful in the treatment of rheumatoid arthritis. Rheumatology (Oxford) 38:1088-1093.

Lambeau G, Lazdunski M (1999) Receptors for a growing family of secreted phospholipases A2. Trends in pharmacological sciences 20:162-170.

Lambeau G, Gelb MH (2008) Biochemistry and physiology of mammalian secreted phospholipases A2. Annual review of biochemistry 77:495-520.

Lappas M, Permezel M, Georgiou HM, Rice GE (2004) Regulation of phospholipase isozymes by nuclear factor-kappaB in human gestational tissues in vitro. J Clin Endocrinol Metab 89:2365-2372.

Lavrovsky Y, Chatterjee B, Clark RA, Roy AK (2000) Role of redox-regulated transcription factors in inflammation, aging and age-related diseases. Experimental gerontology 35:521-532.

Leitinger N, Watson AD, Hama SY, Ivandic B, Qiao JH, Huber J, Faull KF, Grass DS, Navab M, Fogelman AM, de Beer FC, Lusis AJ, Berliner JA (1999) Role of group II secretory phospholipase A2 in atherosclerosis: 2. Potential involvement of biologically active oxidized phospholipids. Arteriosclerosis, thrombosis, and vascular biology 19:1291-1298.

Levites Y, Amit T, Youdim MB, Mandel S (2002) Involvement of protein kinase C activation and cell survival/ cell cycle genes in green tea polyphenol (-)-epigallocatechin 3-gallate neuroprotective action. The Journal of biological chemistry 277:30574-30580.

Levites Y, Amit T, Mandel S, Youdim MB (2003) Neuroprotection and neurorescue against Abeta toxicity and PKC-dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (-)-epigallocatechin-3-gallate. Faseb J 17:952-954.

Li W, Xia J, Sun GY (1999) Cytokine induction of iNOS and sPLA2 in immortalized astrocytes (DITNC): response to genistein and pyrrolidine dithiocarbamate. J Interferon Cytokine Res 19:121-127.

Lin MK, Farewell V, Vadas P, Bookman AA, Keystone EC, Pruzanski W (1996) Secretory phospholipase A2 as an index of disease activity in rheumatoid arthritis. Prospective double blind study of 212 patients. J Rheumatol 23:1162-1166.

Lin TN, Wang Q, Simonyi A, Chen JJ, Cheung WM, He YY, Xu J, Sun AY, Hsu CY, Sun GY (2004) Induction of secretory phospholipase A2 in reactive astrocytes in response to transient focal cerebral ischemia in the rat brain. J Neurochem 90:637-645.

Liu Q, Kang JH, Zheng RL (2005) NADPH oxidase produces reactive oxygen species and maintains survival of rat astrocytes. Cell biochemistry and function 23:93-100.

Liu Z, Lu X, Wang H, You XJ, Gao QX, Cui YH (2007) Group II subfamily secretory phospholipase A2 enzymes: expression in chronic rhinosinusitis with and without nasal polyps. Allergy 62:999-1006.

Luchtefeld R, Luo R, Stine K, Alt ML, Chernovitz PA, Smith RE (2008) Dose formulation and analysis of diapocynin. J Agric Food Chem 56:301-306.

Lue LF, Walker DG, Rogers J (2001a) Modeling microglial activation in Alzheimer's disease with human postmortem microglial cultures. Neurobiol Aging 22:945-956.

Lue LF, Rydel R, Brigham EF, Yang LB, Hampel H, Murphy GM, Jr., Brachova L, Yan SD, Walker DG, Shen Y, Rogers J (2001b) Inflammatory repertoire of Alzheimer's disease and nondemented elderly microglia in vitro. Glia 35:72-79.

Macchioni L, Corazzi L, Nardicchi V, Mannucci R, Arcuri C, Porcellati S, Sposini T, Donato R, Goracci G (2004) Rat brain cortex mitochondria release group II

secretory phospholipase A(2) under reduced membrane potential. J Biol Chem 279:37860-37869.

Mallat Z, Benessiano J, Simon T, Ederhy S, Sebella-Arguelles C, Cohen A, Huart V, Wareham NJ, Luben R, Khaw KT, Tedgui A, Boekholdt SM (2007) Circulating secretory phospholipase A2 activity and risk of incident coronary events in healthy men and women: the EPIC-Norfolk study. Arteriosclerosis, thrombosis, and vascular biology 27:1177-1183.

Mancuso C, Bates TE, Butterfield DA, Calafato S, Cornelius C, De Lorenzo A, Dinkova Kostova AT, Calabrese V (2007) Natural antioxidants in Alzheimer's disease. Expert Opin Investig Drugs 16:1921-1931.

Mandel S, Weinreb O, Amit T, Youdim MB (2004) Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (-)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. Journal of neurochemistry 88:1555-1569.

Markesbery WR (1997) Oxidative stress hypothesis in Alzheimer's disease. Free radical biology & medicine 23:134-147.

Masuda S, Yamamoto K, Hirabayashi T, Ishikawa Y, Ishii T, Kudo I, Murakami M (2008) Human group III secreted phospholipase A2 promotes neuronal outgrowth and survival. The Biochemical journal 409:429-438.

Masuda S, Murakami M, Takanezawa Y, Aoki J, Arai H, Ishikawa Y, Ishii T, Arioka M, Kudo I (2005) Neuronal expression and neuritogenic action of group X secreted phospholipase A2. The Journal of biological chemistry 280:23203-23214.

Mathisen GH, Thorkildsen IH, Paulsen RE (2007) Secretory PLA2-IIA and ROS generation in peripheral mitochondria are critical for neuronal death. Brain research 1153:43-51.

McGeer EG, McGeer PL (2003) Inflammatory processes in Alzheimer's disease. Prog Neuropsychopharmacol Biol Psychiatry 27:741-749.

McGeer EG, Klegeris A, McGeer PL (2005) Inflammation, the complement system and the diseases of aging. Neurobiol Aging 26 Suppl 1:94-97.

Miller RL, Sun GY, Sun AY (2007) Cytotoxicity of paraquat in microglial cells: Involvement of PKCdelta- and ERK1/2-dependent NADPH oxidase. Brain research 1167:129-139.

Min KJ, Pyo HK, Yang MS, Ji KA, Jou I, Joe EH (2004) Gangliosides activate microglia via protein kinase C and NADPH oxidase. Glia 48:197-206.

Molloy GY, Rattray M, Williams RJ (1998) Genes encoding multiple forms of phospholipase A2 are expressed in rat brain. Neuroscience letters 258:139-142.

Moreira PI, Siedlak SL, Aliev G, Zhu X, Cash AD, Smith MA, Perry G (2005) Oxidative stress mechanisms and potential therapeutics in Alzheimer disease. J Neural Transm 112:921-932.

Morris JC, Storandt M, McKeel DW, Jr., Rubin EH, Price JL, Grant EA, Berg L (1996) Cerebral amyloid deposition and diffuse plaques in "normal" aging: Evidence for presymptomatic and very mild Alzheimer's disease. Neurology 46:707-719.

Moses GS, Jensen MD, Lue LF, Walker DG, Sun AY, Simonyi A, Sun GY (2006) Secretory PLA2-IIA: a new inflammatory factor for Alzheimer's disease. Journal of neuroinflammation [electronic resource] 3:28.

Mounier CM, Wendum D, Greenspan E, Flejou JF, Rosenberg DW, Lambeau G (2008) Distinct expression pattern of the full set of secreted phospholipases A2 in human colorectal adenocarcinomas: sPLA2-III as a biomarker candidate. Br J Cancer 98:587-595.

Mounier CM, Ghomashchi F, Lindsay MR, James S, Singer AG, Parton RG, Gelb MH (2004) Arachidonic acid release from mammalian cells transfected with human groups IIA and X secreted phospholipase A(2) occurs predominantly during the secretory process and with the involvement of cytosolic phospholipase A(2)-alpha. The Journal of biological chemistry 279:25024-25038.

Moynagh PN (2005) The interleukin-1 signalling pathway in astrocytes: a key contributor to inflammation in the brain. J Anat 207:265-269.

Mrak RE, Griffin WS (2005) Glia and their cytokines in progression of neurodegeneration. Neurobiol Aging 26:349-354.

Mrak RE, Sheng JG, Griffin WS (1996) Correlation of astrocytic S100 beta expression with dystrophic neurites in amyloid plaques of Alzheimer's disease. J Neuropathol Exp Neurol 55:273-279.

Murakami M, Kudo I (2002a) Phospholipase A2. J Biochem 131:285-292.

Murakami M, Kudo I (2002b) Phospholipase A2. J Biochem (Tokyo) 131:285-292.

Murakami M, Nakatani Y, Atsumi G, Inoue K, Kudo I (1997) Regulatory functions of phospholipase A2. Critical reviews in immunology 17:225-283.

Murakami M, Kambe T, Shimbara S, Yamamoto S, Kuwata H, Kudo I (1999) Functional association of type IIA secretory phospholipase A(2) with the glycosylphosphatidylinositol-anchored heparan sulfate proteoglycan in the

cyclooxygenase-2-mediated delayed prostanoid-biosynthetic pathway. The Journal of biological chemistry 274:29927-29936.

Murakami M, Masuda S, Shimbara S, Ishikawa Y, Ishii T, Kudo I (2005) Cellular distribution, post-translational modification, and tumorigenic potential of human group III secreted phospholipase A(2). The Journal of biological chemistry 280:24987-24998.

Murakami M, Shimbara S, Kambe T, Kuwata H, Winstead MV, Tischfield JA, Kudo I (1998) The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. The Journal of biological chemistry 273:14411-14423.

Murakami M, Yoshihara K, Shimbara S, Lambeau G, Singer A, Gelb MH, Sawada M, Inagaki N, Nagai H, Kudo I (2002a) Arachidonate release and eicosanoid generation by group IIE phospholipase A(2). Biochemical and biophysical research communications 292:689-696.

Murakami M, Yoshihara K, Shimbara S, Sawada M, Inagaki N, Nagai H, Naito M, Tsuruo T, Moon TC, Chang HW, Kudo I (2002b) Group IID heparin-binding secretory phospholipase A(2) is expressed in human colon carcinoma cells and human mast cells and up-regulated in mouse inflammatory tissues. Eur J Biochem 269:2698-2707.

Murakami M, Masuda S, Shimbara S, Bezzine S, Lazdunski M, Lambeau G, Gelb MH, Matsukura S, Kokubu F, Adachi M, Kudo I (2003) Cellular arachidonate-releasing function of novel classes of secretory phospholipase A2s (groups III and XII). The Journal of biological chemistry 278:10657-10667.

Murakami M, Yoshihara K, Shimbara S, Lambeau G, Gelb MH, Singer AG, Sawada M, Inagaki N, Nagai H, Ishihara M, Ishikawa Y, Ishii T, Kudo I (2002c) Cellular arachidonate-releasing function and inflammation-associated expression of group IIF secretory phospholipase A2. The Journal of biological chemistry 277:19145-19155.

Nakashima S, Kitamoto K, Arioka M (2004) The catalytic activity, but not receptor binding, of sPLA2s plays a critical role for neurite outgrowth induction in PC12 cells. Brain research 1015:207-211.

Nguyen MD, Julien JP, Rivest S (2002) Innate immunity: the missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci 3:216-227.

Ni Z, Okeley NM, Smart BP, Gelb MH (2006) Intracellular actions of group IIA secreted phospholipase A2 and group IVA cytosolic phospholipase A2 contribute to arachidonic acid release and prostaglandin production in rat gastric mucosal cells

and transfected human embryonic kidney cells. The Journal of biological chemistry 281:16245-16255.

Niessen HW, Krijnen PA, Visser CA, Meijer CJ, Erik Hack C (2003) Type II secretory phospholipase A2 in cardiovascular disease: a mediator in atherosclerosis and ischemic damage to cardiomyocytes? Cardiovasc Res 60:68-77.

Nijmeijer R, Willemsen M, Meijer CJ, Visser CA, Verheijen RH, Gottlieb RA, Hack CE, Niessen HW (2003) Type II secretory phospholipase A2 binds to ischemic flip-flopped cardiomyocytes and subsequently induces cell death. Am J Physiol Heart Circ Physiol 285:H2218-2224.

Nishikawa H, Wakano K, Kitani S (2007) Inhibition of NADPH oxidase subunits translocation by tea catechin EGCG in mast cell. Biochemical and biophysical research communications 362:504-509.

Noh KM, Koh JY (2000) Induction and activation by zinc of NADPH oxidase in cultured cortical neurons and astrocytes. J Neurosci 20:RC111.

Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, Smith MA (1999) RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. J Neurosci 19:1959-1964.

Ohtsuki M, Taketomi Y, Arata S, Masuda S, Ishikawa Y, Ishii T, Takanezawa Y, Aoki J, Arai H, Yamamoto K, Kudo I, Murakami M (2006) Transgenic expression of group V, but not group X, secreted phospholipase A2 in mice leads to neonatal lethality because of lung dysfunction. The Journal of biological chemistry 281:36420-36433.

Oka S, Arita H (1991) Inflammatory factors stimulate expression of group II phospholipase A2 in rat cultured astrocytes. Two distinct pathways of the gene expression. J Biol Chem 266:9956-9960.

Oslund RC, Cermak N, Gelb MH (2008) Highly specific and broadly potent inhibitors of mammalian secreted phospholipases A2. Journal of medicinal chemistry 51:4708-4714.

Packer L (1998) alpha-Lipoic acid: a metabolic antioxidant which regulates NF-kappa B signal transduction and protects against oxidative injury. Drug metabolism reviews 30:245-275.

Pawate S, Shen Q, Fan F, Bhat NR (2004) Redox regulation of glial inflammatory response to lipopolysaccharide and interferongamma. J Neurosci Res 77:540-551.

Peng T, Lu X, Feng Q (2005) Pivotal role of gp91phox-containing NADH oxidase in lipopolysaccharide-induced tumor necrosis factor-alpha expression and myocardial depression. Circulation 111:1637-1644.

Pike CJ, Cummings BJ, Cotman CW (1995) Early association of reactive astrocytes with senile plaques in Alzheimer's disease. Exp Neurol 132:172-179.

Polazzi E, Contestabile A (2002) Reciprocal interactions between microglia and neurons: from survival to neuropathology. Rev Neurosci 13:221-242.

Probst A, Langui D, Ulrich J (1991) Alzheimer's disease: a description of the structural lesions. Brain Pathol 1:229-239.

Ranaivo HR, Craft JM, Hu W, Guo L, Wing LK, Van Eldik LJ, Watterson DM (2006) Glia as a therapeutic target: selective suppression of human amyloid-beta-induced upregulation of brain proinflammatory cytokine production attenuates neurodegeneration. J Neurosci 26:662-670.

Reid RC (2005) Inhibitors of secretory phospholipase A2 group IIA. Curr Med Chem 12:3011-3026.

Reynolds A, Laurie C, Mosley RL, Gendelman HE (2007) Oxidative stress and the pathogenesis of neurodegenerative disorders. Int Rev Neurobiol 82:297-325.

Rosenberger TA, Villacreses NE, Hovda JT, Bosetti F, Weerasinghe G, Wine RN, Harry GJ, Rapoport SI (2004) Rat brain arachidonic acid metabolism is increased by a 6-day intracerebral ventricular infusion of bacterial lipopolysaccharide. Journal of neurochemistry 88:1168-1178.

Saha RN, Pahan K (2006) Regulation of inducible nitric oxide synthase gene in glial cells. Antioxid Redox Signal 8:929-947.

Samuelsson M, Fisher L, Iverfeldt K (2005) beta-Amyloid and interleukin-1beta induce persistent NF-kappaB activation in rat primary glial cells. Int J Mol Med 16:449-453.

Satake Y, Diaz BL, Balestrieri B, Lam BK, Kanaoka Y, Grusby MJ, Arm JP (2004) Role of group V phospholipase A2 in zymosan-induced eicosanoid generation and vascular permeability revealed by targeted gene disruption. The Journal of biological chemistry 279:16488-16494.

Scholz K, Vlachojannis GJ, Spitzer S, Schini-Kerth V, Van Den Bosch H, Kaszkin M, Pfeilschifter J (1999) Modulation of cytokine-induced expression of secretory phospholipase A2-type IIA by protein kinase C in rat renal mesangial cells. Biochemical pharmacology 58:1751-1758.

Schreck R, Rieber P, Baeuerle PA (1991) Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF-kappa B transcription factor and HIV-1. The EMBO journal 10:2247-2258.

Schreck R, Meier B, Mannel DN, Droge W, Baeuerle PA (1992) Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. The Journal of experimental medicine 175:1181-1194.

Schwab C, McGeer PL (2008) Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders. J Alzheimers Dis 13:359-369.

Scott DL, White SP, Otwinowski Z, Yuan W, Gelb MH, Sigler PB (1990) Interfacial catalysis: the mechanism of phospholipase A2. Science 250:1541-1546.

Selkoe DJ (1996) Amyloid beta-protein and the genetics of Alzheimer's disease. The Journal of biological chemistry 271:18295-18298.

Shaftel SS, Kyrkanides S, Olschowka JA, Miller JN, Johnson RE, O'Banion MK (2007) Sustained hippocampal IL-1 beta overexpression mediates chronic neuroinflammation and ameliorates Alzheimer plaque pathology. The Journal of clinical investigation 117:1595-1604.

Shelat PB, Chalimoniuk M, Wang JH, Strosznajder JB, Lee JC, Sun AY, Simonyi A, Sun GY (2008) Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons. Journal of neurochemistry 106:45-55.

Shirai Y, Ito M (2004) Specific differential expression of phospholipase A2 subtypes in rat cerebellum. J Neurocytol 33:297-307.

Silvestrelli G, Lanari A, Parnetti L, Tomassoni D, Amenta F (2006) Treatment of Alzheimer's disease: From pharmacology to a better understanding of disease pathophysiology. Mech Ageing Dev 127:148-157.

Simonian NA, Coyle JT (1996) Oxidative stress in neurodegenerative diseases. Annual review of pharmacology and toxicology 36:83-106.

Son TG, Camandola S, Mattson MP (2008) Hormetic Dietary Phytochemicals. Neuromolecular medicine.

Stefanska J, Pawliczak R (2008) Apocynin: molecular aptitudes. Mediators Inflamm 2008:106507.

Steffen Y, Gruber C, Schewe T, Sies H (2008) Mono-O-methylated flavanols and other flavonoids as inhibitors of endothelial NADPH oxidase. Arch Biochem Biophys 469:209-219.

Stephenson D, Rash K, Smalstig B, Roberts E, Johnstone E, Sharp J, Panetta J, Little S, Kramer R, Clemens J (1999) Cytosolic phospholipase A2 is induced in reactive glia following different forms of neurodegeneration. Glia 27:110-128.

Stephenson DT, Lemere CA, Selkoe DJ, Clemens JA (1996) Cytosolic phospholipase A2 (cPLA2) immunoreactivity is elevated in Alzheimer's disease brain. Neurobiol Dis 3:51-63.

Stolk J, Hiltermann TJ, Dijkman JH, Verhoeven AJ (1994) Characteristics of the inhibition of NADPH oxidase activation in neutrophils by apocynin, a methoxy-substituted catechol. American journal of respiratory cell and molecular biology 11:95-102.

Streit WJ (2004) Microglia and Alzheimer's disease pathogenesis. J Neurosci Res 77:1-8.

Stuchbury G, Munch G (2005) Alzheimer's associated inflammation, potential drug targets and future therapies. J Neural Transm 112:429-453.

Suh SW, Gum ET, Hamby AM, Chan PH, Swanson RA (2007) Hypoglycemic neuronal death is triggered by glucose reperfusion and activation of neuronal NADPH oxidase. The Journal of clinical investigation 117:910-918.

Sun AY, Wang Q, Simonyi A, Sun GY (2008a) Botanical phenolics and brain health. Neuromolecular Med 10:259-274.

Sun GY, Hu ZY (1995) Stimulation of phospholipase A2 expression in rat cultured astrocytes by LPS, TNF alpha and IL-1 beta. Progress in brain research 105:231-238.

Sun GY, Horrocks LA, Farooqui AA (2007) The roles of NADPH oxidase and phospholipases A2 in oxidative and inflammatory responses in neurodegenerative diseases. Journal of neurochemistry 103:1-16.

Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. J Lipid Res 45:205-213.

Sun GY, Xu J, Jensen MD, Yu S, Wood WG, Gonzalez FA, Simonyi A, Sun AY, Weisman GA (2005) Phospholipase A2 in astrocytes: responses to oxidative stress, inflammation, and G protein-coupled receptor agonists. Mol Neurobiol 31:27-41.

Sun HN, Kim SU, Lee MS, Kim SK, Kim JM, Yim M, Yu DY, Lee DS (2008b) Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent activation of phosphoinositide 3-kinase and p38 mitogen-activated protein kinase

signal pathways is required for lipopolysaccharide-induced microglial phagocytosis. Biol Pharm Bull 31:1711-1715.

Suzuki K, Hino M, Kutsuna H, Hato F, Sakamoto C, Takahashi T, Tatsumi N, Kitagawa S (2001) Selective activation of p38 mitogen-activated protein kinase cascade in human neutrophils stimulated by IL-1beta. J Immunol 167:5940-5947.

Suzuki N, Ishizaki J, Yokota Y, Higashino K, Ono T, Ikeda M, Fujii N, Kawamoto K, Hanasaki K (2000) Structures, enzymatic properties, and expression of novel human and mouse secretory phospholipase A(2)s. The Journal of biological chemistry 275:5785-5793.

Svensson CI, Lucas KK, Hua XY, Powell HC, Dennis EA, Yaksh TL (2005) Spinal phospholipase A2 in inflammatory hyperalgesia: role of the small, secretory phospholipase A2. Neuroscience 133:543-553.

Sweatt JD (2001) The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. Journal of neurochemistry 76:1-10.

Taketo MM, Sonoshita M (2002) Phospolipase A2 and apoptosis. Biochim Biophys Acta 1585:72-76.

Thwin MM, Ong WY, Fong CW, Sato K, Kodama K, Farooqui AA, Gopalakrishnakone P (2003) Secretory phospholipase A2 activity in the normal and kainate injected rat brain, and inhibition by a peptide derived from python serum. Exp Brain Res 150:427-433.

Tietge UJ, Pratico D, Ding T, Funk CD, Hildebrand RB, Van Berkel T, Van Eck M (2005) Macrophage-specific expression of group IIA sPLA2 results in accelerated atherogenesis by increasing oxidative stress. Journal of lipid research 46:1604-1614.

Tischfield JA (1997) A reassessment of the low molecular weight phospholipase A2 gene family in mammals. J Biol Chem 272:17247-17250.

Tischfield JA, Xia YR, Shih DM, Klisak I, Chen J, Engle SJ, Siakotos AN, Winstead MV, Seilhamer JJ, Allamand V, Gyapay G, Lusis AJ (1996) Low-molecular-weight, calcium-dependent phospholipase A2 genes are linked and map to homologous chromosome regions in mouse and human. Genomics 32:328-333.

Tong W, Shah D, Xu J, Diehl JA, Hans A, Hannink M, Sun GY (1999) Involvement of lipid mediators on cytokine signaling and induction of secretory phospholipase A2 in immortalized astrocytes (DITNC). J Mol Neurosci 12:89-99.

Touqui L, Alaoui-El-Azher M (2001) Mammalian secreted phospholipases A2 and their pathophysiological significance in inflammatory diseases. Curr Mol Med 1:739-754.

Vadas P, Browning J, Edelson J, Pruzanski W (1993) Extracellular phospholipase A2 expression and inflammation: the relationship with associated disease states. J Lipid Mediat 8:1-30.

Valentin E, Lambeau G (2000) Increasing molecular diversity of secreted phospholipases A(2) and their receptors and binding proteins. Biochim Biophys Acta 1488:59-70.

Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G (1999) On the diversity of secreted phospholipases A(2). Cloning, tissue distribution, and functional expression of two novel mouse group II enzymes. The Journal of biological chemistry 274:31195-31202.

Valentin E, Ghomashchi F, Gelb MH, Lazdunski M, Lambeau G (2000) Novel human secreted phospholipase A(2) with homology to the group III bee venom enzyme. The Journal of biological chemistry 275:7492-7496.

Van den Worm E, Beukelman CJ, Van den Berg AJ, Kroes BH, Labadie RP, Van Dijk H (2001) Effects of methoxylation of apocynin and analogs on the inhibition of reactive oxygen species production by stimulated human neutrophils. Eur J Pharmacol 433:225-230.

Varadarajan S, Yatin S, Aksenova M, Butterfield DA (2000) Review: Alzheimer's amyloid beta-peptide-associated free radical oxidative stress and neurotoxicity. J Struct Biol 130:184-208.

Verheij HM, Slotboom AJ, de Haas GH (1981) Structure and function of phospholipase A2. Rev Physiol Biochem Pharmacol 91:91-203.

Vervoordeldonk MJ, Pineda Torra IM, Aarsman AJ, van den Bosch H (1996) Aspirin inhibits expression of the interleukin-1beta-inducible group II phospholipase A2. FEBS letters 397:108-112.

Walker DG, Lue LF (2005) Investigations with cultured human microglia on pathogenic mechanisms of Alzheimer's disease and other neurodegenerative diseases. J Neurosci Res 81:412-425.

Walker DG, Lue LF, Beach TG (2001) Gene expression profiling of amyloid beta peptide-stimulated human post-mortem brain microglia. Neurobiol Aging 22:957-966.

Walker DG, Link J, Lue LF, Dalsing-Hernandez JE, Boyes BE (2006) Gene expression changes by amyloid {beta} peptide-stimulated human postmortem brain microglia identify activation of multiple inflammatory processes. J Leukoc Biol 79:596-610.

Wang G, Daniel BM, DeCoster MA (2005) Role of nitric oxide in regulating secreted phospholipase A2 release from astrocytes. Neuroreport 16:1345-1350.

Wang Q, Yu S, Simonyi A, Rottinghaus G, Sun GY, Sun AY (2004) Resveratrol protects against neurotoxicity induced by kainic acid. Neurochemical research 29:2105-2112.

Wang Q, Tompkins KD, Simonyi A, Korthuis RJ, Sun AY, Sun GY (2006) Apocynin protects against global cerebral ischemia-reperfusion-induced oxidative stress and injury in the gerbil hippocampus. Brain research 1090:182-189.

Wang Q, Xu J, Rottinghaus GE, Simonyi A, Lubahn D, Sun GY, Sun AY (2002) Resveratrol protects against global cerebral ischemic injury in gerbils. Brain research 958:439-447.

Wang Q, Smith RE, Luchtefeld R, Sun AY, Simonyi A, Luo R, Sun GY (2008) Bioavailability of apocynin through its conversion to glycoconjugate but not to diapocynin. Phytomedicine 15:496-503.

Wei S, Ong WY, Thwin MM, Fong CW, Farooqui AA, Gopalakrishnakone P, Hong W (2003) Group IIA secretory phospholipase A2 stimulates exocytosis and neurotransmitter release in pheochromocytoma-12 cells and cultured rat hippocampal neurons. Neuroscience 121:891-898.

Wijewickrama GT, Kim JH, Kim YJ, Abraham A, Oh Y, Ananthanarayanan B, Kwatia M, Ackerman SJ, Cho W (2006a) Systematic evaluation of transcellular activities of secretory phospholipases A2. High activity of group V phospholipases A2 to induce eicosanoid biosynthesis in neighboring inflammatory cells. The Journal of biological chemistry 281:10935-10944.

Wijewickrama GT, Albanese A, Kim YJ, Oh YS, Murray PS, Takayanagi R, Tobe T, Masuda S, Murakami M, Kudo I, Ucker DS, Murray D, Cho W (2006b) Unique membrane interaction mode of group IIF phospholipase A2. The Journal of biological chemistry 281:32741-32754.

Wu CY, Hsieh HL, Jou MJ, Yang CM (2004) Involvement of p42/p44 MAPK, p38 MAPK, JNK and nuclear factor-kappa B in interleukin-1beta-induced matrix metalloproteinase-9 expression in rat brain astrocytes. Journal of neurochemistry 90:1477-1488.

Xu J, Weng YI, Simonyi A, Krugh BW, Liao Z, Weisman GA, Sun GY (2002) Role of PKC and MAPK in cytosolic PLA2 phosphorylation and arachadonic acid release in primary murine astrocytes. J Neurochem 83:259-270.

Xu J, Chalimoniuk M, Shu Y, Simonyi A, Sun AY, Gonzalez FA, Weisman GA, Wood WG, Sun GY (2003) Prostaglandin E2 production in astrocytes: regulation by cytokines, extracellular ATP, and oxidative agents. Prostaglandins Leukot Essent Fatty Acids 69:437-448.

Yagami T, Ueda K, Sakaeda T, Itoh N, Sakaguchi G, Okamura N, Hori Y, Fujimoto M (2004) Protective effects of a selective L-type voltage-sensitive calcium channel blocker, S-312-d, on neuronal cell death. Biochem Pharmacol 67:1153-1165.

Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Takasu N, Tanaka K, Gemba T, Hori Y (2002a) Human group IIA secretory phospholipase A2 induces neuronal cell death via apoptosis. Mol Pharmacol 61:114-126.

Yagami T, Ueda K, Asakura K, Hata S, Kuroda T, Sakaeda T, Kishino J, Sakaguchi G, Itoh N, Hori Y (2002b) Group IB secretory phospholipase A(2)induces cell death in the cultured cortical neurons: a possible involvement of its binding sites. Brain research 949:197-201.

Yang CS, Lee HM, Lee JY, Kim JA, Lee SJ, Shin DM, Lee YH, Lee DS, El-Benna J, Jo EK (2007a) Reactive oxygen species and p47phox activation are essential for the Mycobacterium tuberculosis-induced pro-inflammatory response in murine microglia. J Neuroinflammation 4:27.

Yang MS, Min KJ, Joe E (2007b) Multiple mechanisms that prevent excessive brain inflammation. J Neurosci Res 85:2298-2305.

Zaleska MM, Floyd RA (1985) Regional lipid peroxidation in rat brain in vitro: possible role of endogenous iron. Neurochemical research 10:397-410.

Zaveri NT (2006) Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications. Life Sci 78:2073-2080.

SUMMARY AND FUTURE DIRECTIONS

Summary

Alzheimer's disease has many aspects to its pathology, including inflammation and oxidative stress. The purpose of these research studies is to identify the role of sPLA2-IIA, based on the premise that sPLA2-IIA is upregulated by conditions in AD (cytokine induction, ROS generation), and that exogenous sPLA2-IIA has the capability to cause neuronal injury. To begin, we first needed to look at the localization and regulation of sPLA2-IIA in the AD brain.

With our first study (Chapter 2) we were able to identify an increase in sPLA2-IIA mRNA in hippocampus of AD brains, where most pathological changes occur. In cerebellum, no such increase in sPLA2-IIA mRNA was observed. With these results, we identified the localization to astrocytes of sPLA2-IIA protein, and found it higher in AD brains than non-dementia (ND) brains. Our next study identified the proximity of these reactive astrocytes with increased sPLA2-IIA near amyloid fibrils, suggesting that A-beta may participate in the induction of sPLA2-IIA mRNA through factors affecting the reactivity of the astrocytes. A-beta alone was capable of inducing sPLA2-IIA mRNA in cultured astrocytes.

Our second study (Chapter 3) took another step in our overall purpose, looking at the oxidative stress aspect of the AD pathology. We hypothesized that sPLA2-IIA was connected to ROS through NADPH oxidase. Our first studies looked at several kinases involved in the activation of NADPH oxidase, and we tested the corresponding cytokine-stimulated upregulation of sPLA2-IIA mRNA while we inhibited those pathways. PI3-kinase and ERK1/2, both involved in NADPH oxidase activation, were also playing a

role in sPLA2-IIA mRNA upregulation by IL-1b. Taking it a step further, we investigated an NADPH oxidase inhibitor, apocynin, and its corresponding effect on sPLA2-IIA, with the same results, an inhibition of sPLA2-IIA mRNA induction. Now with results of experiments involving kinases for the activation of NADPH oxidase, and an inhibitor of NADPH oxidase itself, we wanted to test the effects of two botanical polyphenols that have anti-oxidant properties. Resveratrol and epigallocatechin gallate (EGCG) were both able to affect NADPH oxidation, as evidenced by less p67phox translocation to the membrane. The next test then was to see the effect of these same compounds on the cytokine induction of sPLA2-IIA, and both resveratrol and EGCG were able to inhibit its induction.

There are a few limitations to these studies to point out. The study would be much stronger if we had a more direct NADPH oxidase inhibitor. Apocynin is known on a chemical level to inhibit NADPH oxidase, but it also may have other effects as it is known also to be an anti-oxidant. In addition, assays for measuring ROS in cells were not effective, most likely due to the amount of ROS generated not being proportional to the precision of the assay. Experiments looking at ROS on a microscopic level didn't generate reliable data from experiment to experiment with the equipment we had available.

The third aspect of my doctoral work included a fellowship in bioinformatics from the NLM studying, training on, and developing web services. Web services is one of the strongest solutions to the problem of data integration between services and researchers.

Workflows take web services and make them into a repeatable, multi-service process. In a workflow, software combines (or allows the user to combine) a series of coordinated services to create a data analysis pipeline. A workflow starts with an input, processes this input with one or more services, and then returns with the output. With the ability to discover web services with a registry and programmatically connect to these web services based on the input and output of each service, workflows can be designed and run to process scientific data. Ideally workflows can be saved, reused, and shared, making scientific data integration possible for even non-technical users.

For researchers of any computer skill level, the easiest way to access web services is to use existing applications and tools, like Taverna (http://mygrid.org.uk) or BioMOBY (http://biomoby.org). These tools make it simple to find and use available services, and typically output the data in a file or format for viewing. Many tools are available for simply accessing a single service, while only a few simplify the use of workflows to connect these web services systematically.

One of the additional benefits of web services and workflows is the opportunity for data discovery. A researcher with data in hand could query a registry for services that take their data and show all possible services and possible outputs, giving the researchers a simple way to expand the use of their data beyond typical data analysis techniques used within the laboratory. With workflows being easy to reuse and share, the publication and sharing of a workflow is made possible for distribution and use within a multi-collaborative study, as well as for other research groups to repeat and confirm experiments.

The availability of web services and the development of neuroscience-specific ontologies should provide a means for improved data and service integration within the neuroscience discipline and other fields of study in the sciences. With the ongoing development of web services making use of protocols compatible with open projects like BioMOBY or myGrid, more opportunites for data integration, sharing, and discovery are on the horizon.

Future Directions

The results of these studies have brought interesting and exciting results that give insight into the internal workings of astrocytes and sPLA2-IIA under these conditions. The next logical step, working on the basis of our studies, is to look at the activity and effect of sPLA2-IIA. Studies could include quantifying the secretion of sPLA2-IIA from astrocytes, and identifying the activity levels of the protein as it is secreted, and as it associates with other cell types. Performing experiments with co-cultures of astrocytes and neurons, and investigating the effect of reactive astrocytes secreting sPLA2-IIA in proximity to neurons in a perturbed state (similar to AD) would bring insight into whether sPLA2-IIA from reactive astrocytes could cause neuronal injury. The difficulty lies in being able to suppress sPLA2-IIA in the control, but it could be realized using new techniques such as RNAi.

From the perspective of oxidative stress, future studies could measure ROS production and characterize the role of ROS itself in sPLA2-IIA induction. The activation of NADPH oxidase and inhibition correlate with that of sPLA2-IIA mRNA, but the

question remains whether this is attributed to similar pathways and cross-talk, or whether ROS is a signaling molecule that affects factors involved in the upregulation of sPLA2-IIA.

New advancements in animal models, such as the Samaritan Alzheimer's Rat Model, would also give rise to opportunities for investigating the role of sPLA2-IIA in Alzheimer's disease. Studies could identify the expression of sPLA2-IIA in this model, and correlate mRNA or protein expression with reactive astrocytes, A-β deposition, and other pathological indicators. The use of various sPLA2 inhibitors targeted to areas of high astrocyte reactivity and susceptibility to neuronal damage could help isolate the role of sPLA2 in the progression of the model, both molecular and behavioral.

Vita

Michael Daniel Jensen was born in Portland, Oregon, USA on May 30, 1979 to Daniel O. and Beverly A. Jensen. After attending Prairie High School and Clark College (Vancouver, Washington, USA) he studied at Brigham Young University (Provo, Utah, USA) and graduated with a B.S. in Nutritional Sciences in 2002. He has been a Doctoral Student since 2002 with the University of Missouri-Columbia (Columbia, Missouri, USA) in the department of Biochemistry under the supervision of Dr. Grace Y. Sun.

Publications

Jensen MD, Sheng W, Simonyi A, Johnson GS, Sun AY, Sun GY (2009) Involvement of oxidative pathways in cytokine-induced secretory phospholipase A2-IIA in astrocytes. Neurochem Int. 55 (2009) 362-368.

Moses GS, Jensen MD, Lue LF, Walker DG, Sun AY, Simonyi A, Sun GY (2006) Secretory PLA2-IIA: a new inflammatory factor for Alzheimer's disease. Journal of neuroinflammation [electronic resource] 3:28.

Sun GY, Xu J, Jensen MD, Simonyi A (2004) Phospholipase A2 in the central nervous system: implications for neurodegenerative diseases. Journal of lipid research 45:205-213.

Sun GY, Xu J, Jensen MD, Yu S, Wood WG, Gonzalez FA, Simonyi A, Sun AY, Weisman GA (2005) Phospholipase A2 in astrocytes: responses to oxidative stress, inflammation, and G protein-coupled receptor agonists. Mol Neurobiol 31:27-41.

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