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Lipid Oxidation and Peroxidation in CNS Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities

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Abstract

Reactive oxygen species (ROS) are produced at low levels in mammalian cells by various metabolic processes, such as oxidative phosphorylation by the mitochondrial respiratory chain, NAD(P)H oxidases, and arachidonic acid oxidative metabolism. To maintain physiological redox balance, cells have endogenous antioxidant defenses regulated at the transcriptional level by Nrf2/ARE. Oxidative stress results when ROS production exceeds the cell's ability to detoxify ROS. Overproduction of ROS damages cellular components, including lipids, leading to decline in physiological function and cell death. Reaction of ROS with lipids produces oxidized phospholipids, which give rise to 4-hydroxynonenal, 4-oxo-2-nonenal, and acrolein. The brain is susceptible to oxidative damage due to its high lipid content and oxygen consumption. Neurodegenerative diseases (AD, ALS, bipolar disorder, epilepsy, Friedreich's ataxia, HD, MS, NBIA, NPC, PD, peroxisomal disorders, schizophrenia, Wallerian degeneration, Zellweger syndrome) and CNS traumas (stroke, TBI, SCI) are problems of vast clinical importance. Free iron can react with H_2O_2 *via* the Fenton reaction, a primary cause of lipid peroxidation, and may be of particular importance for these CNS injuries and disorders. Cholesterol is an important regulator of lipid organization and the precursor for neurosteroid biosynthesis. Atherosclerosis, the major risk factor for ischemic stroke, involves accumulation of oxidized LDL in the arteries, leading to foam cell formation and plaque development. This review will discuss the role of lipid oxidation/peroxidation in various CNS injuries/disorders. *Antioxid Redox Signal.* 12, 125–169.

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I. Introduction

Neurodegenerative diseases, mental disorders, stroke, and CNS traumas are problems of vast clinical importance. The crucial role of lipid oxidation and peroxidation through reactive oxygen species (ROS) in tissue pathophysiology is demonstrated by the many neurological disorders, including bipolar disorder and schizophrenia, and neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), multiple sclerosis (MS), Niemann–Pick C (NPC), and Huntington's (HD) diseases, Friedreich's ataxia, infantile neuroaxonal degeneration (INAD), and neurodegeneration with brain iron accumulation (NBIA), and Zellweger syndrome that involve deregulated lipid metabolism (Fig. 1) (4, 5). Oxidative stress and lipid peroxidation are also believed to be key events that contribute to CNS injuries such as stroke, traumatic brain injury (TBI), and spinal cord injury (SCI) (6, 10). Since the discovery of oxidized phospholipids and their role in modulation of inflammation in cardiovascular diseases, the importance of phospholipid oxidation products in several CNS pathologies has been identified. Accumulation of oxidized phospholipids causes immunomodulation and may lead to autoimmune diseases such as MS. The role of oxidized phospholipids has been suggested in neurological disorders such as AD (173), PD (292), MS (276), and CNS injuries, for example, stroke (7). The formation of bioactive lipid and phospholipid oxidation/peroxidation products occurs in all inflammed and CNS tissue subjected to oxidative stress due to specific pathologies. This review presents the importance of lipid oxidation and peroxidation in various neurodegenerative diseases, as well as in CNS traumas; how these basic research discoveries are leading to clinical translations and paving the route to bench-to-bed concepts; and the feedback/challenges from the translational approach back to basic research.

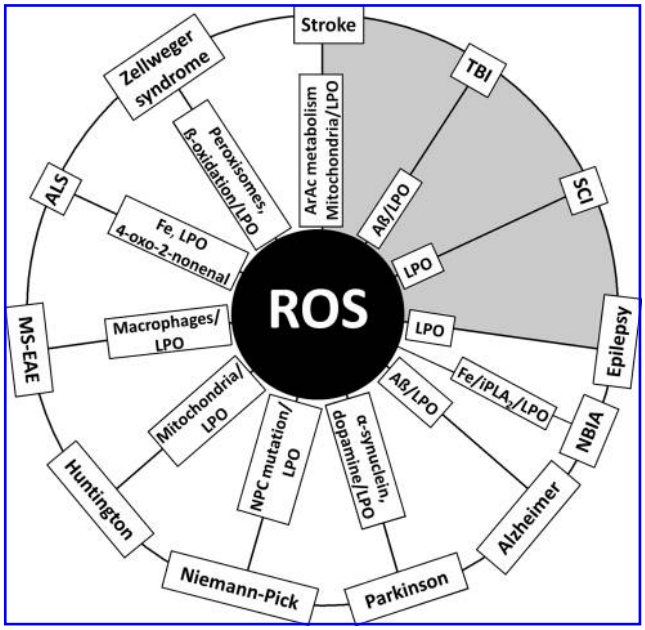


FIG. 1. Reactive oxygen species (ROS) in CNS disorders (*clear*) and injuries (*shaded*). Lipid systems that are affected in CNS injuries and disorders due to activation of phospholipases, impaired lipid synthesis and transport, dysfunctional mitochondria, iron accumulation, and inflammatory cytokine responses. These factors are responsible for ROS generation, lipid oxidation, peroxidation, and one of several contributing factors for the ultimate detrimental effects. $iPLA_2$, Group VIA, calcium independent phospholipase A_2; LPO, lipid peroxidation; ArAc, arachidonic acid.

II. The Biological Membrane Structure and Function

Cellular membranes are composed of glycerophospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI)], sphingolipids [sphingomyelin (SM), ceramide and gangliosides], cholesterol and cholesterol esters, acylglycerols, and fatty acids. The phospholipid bilayer and associated lipids provide not only a permeability barrier but also a structured environment that is essential for the proper functioning of membrane-bound proteins (223). Cholesterol is one of the most important regulators of lipid organization as its structure allows it to fill interstitial spaces between hydrophobic fatty acid chains of phospholipids. The neutral lipids such as PC and SM predominantly reside on the outer or exofacial leaflet, whereas anionic phospholipids PS (exclusively inner leaflet) and PI reside on the inner or cytofacial leaflet of the biological membrane. The transbilayer distribution of cholesterol between the leaflets determines membrane fluidity and can alter the membrane function.

A. Lipid synthesis

1. Fatty acid synthesis. Acetyl–CoA is synthesized in the mitochondrial matrix from ATP and CoA. The first, committed step in fatty acid synthesis is conversion of acetyl CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase. In the cytosol, successive two-carbon units are added by addition of acetyl-CoA to form the 16-carbon saturated fatty acid, palmitic acid (C16:0), the final product of the fatty acid synthase complex, which consists of seven different enzymes and a small protein called acyl carrier protein. Further carbon chain elongation and introduction of double bonds occurs in the endoplasmic reticulum (ER). Desaturases introduce double bonds at specific positions in fatty acid chains. Mammalian cells are unable to produce double bonds at certain locations (*e.g.*, Δ^{12}) and therefore some polyunsaturated fatty acids (PUFA) are dietary essentials. For example, linoleic (C18:2 $\Delta^{9,12}$) and linolenic (C18:3 $\Delta^{6,9,12}$) are not synthesized by mammals and are therefore important dietary requirements for further synthesis of arachidonic (ArAc, C20:4 n-6, $\Delta^{5,8,11,14}$) and docosahexaenoic acid (DHA, C22:6 $\Delta^{4,7,10,13,16,19}$). ArAc is synthesized from γ-linolenic acid by elongation and desaturation.

DHA is derived from the diet or from biosynthesis in the liver. Local synthesis in the brain provides a means for its accumulation in the brain. It is well established that DHA can be synthesized from α-linolenic acid. Among brain cells, DHA synthesis has been demonstrated only in astrocytes; neurons cannot synthesize DHA due to lack of desaturase activity (167). Pharmacological evidence suggests that DHA release from astrocytes is mediated by Ca^{2+}-independent PLA_2 ($iPLA_2$) (123), whereas cytosolic PLA_2 is involved in ArAc release. Since postmitotic neurons do not proliferate; they maintain high levels of membrane PS through DHA enrichment. It is not clear whether loss of DHA from PS will cause PS to flip to the exofacial side of membrane that serves as an apoptotic signal (239). Thus, PS controls apoptosis, and low DHA levels result in lower neural cell PS and an increase in neuronal death.

2. Phospholipid synthesis. Glycerophospholipids are synthesized in four steps: (a) synthesis of the backbone molecule, glycerol-3-phosphate, (b) attachment of fatty acids *via* fatty acyl CoAs yielding phosphatidic acid, (c) dephosphorylation to 1,2-diacylglycerol (DAG), (d) addition of a hydrophilic head group such as phosphocholine, phosphoserine, or phosphoethanolamine. Phosphatidylinositol is formed directly from phosphatidic acid by addition of inositol. In some cases, the phospholipid undergoes alteration (such as methylation of the ethanolamine group to form choline) or exchange of the head group. General assembly of phospholipids is done on the smooth surface of ER and inner mitochondrial membrane.

The first step in sphingolipid synthesis is catalyzed by serine palmitoyl transferase to form dehydrosphingosine (3-ketosphinganine) from palmitoyl-CoA and serine. Dehydrosphingosine is then converted to dihydrosphingosine (sphinganine) and then to dihydroceramide by addition of a fatty acid *via* fatty acyl CoA. Ceramide is synthesized from dihydroceramide by dihydroceramide desaturase. Sphingomyelin (SM) is formed from ceramide by sphingomyelin synthase, which transfers the phosphocholine from PC to ceramide, releasing DAG.

Cardiolipin is a phospholipid that is exclusively limited to the mitochondrial membrane and is essential for proper assembly and functioning of the mitochondrial respiratory chain and oxidative phosphorylation. Mitochondrial cytochrome c release is a critical factor for apoptotic cell death initiated by early cardiolipin oxidation. Early studies demonstrated that ROS produced by mitochondria cause cardiolipin oxidation and a decrease in cardiolipin content, concomitant with loss of

cytochrome c oxidase activity. Oxidative lipidomic studies showed that a pool of cardiolipin-bound cytochrome c can catalyze early cardiolipin peroxidation that facilitates release of cytochrome c into the cytoplasm to trigger an early death pathway that will lead to caspase activation and cell death (155, 156, 257, 259) (Fig. 2). Oxidized cardiolipin was essential for the release of pro-apoptotic factors into the cytosol while non-oxidized cardiolipin was less effective.

Bis(monoacylglycerol)phosphate (lysobisphosphatidic acid, LBPA) is an unusual phospholipid that is detected only in late endosomes, accounts for ~15 mole% of total organelle phospholipids, and also serves as a marker for this organelle. LBPA is a structural isomer of phosphatidylglycerol. While phosphatidylglycerol binds two acyl groups to one glycerol moiety, LBPA binds one acyl group to each glycerol moiety. LBPA is believed to regulate the cholesterol storage capacity of endosomes and might play an important role in Niemann-Pick C disease (63a, 172a). Docosahexaenoic acid (DHA) incorporation into fibroblast LBPA excludes cholesterol from late endosomes (37a). The high capacity of LBPA to incorporate DHA may act as a potential antioxidant and may play an important role in antiphospholipid syndrome (45a). Antiphospholipid syndrome is a disorder of coagulation, which causes blood clots (thrombosis) in both arteries and veins, as well as pregnancy-related complications such as miscarriage or stillbirth, and preterm delivery.

In addition to their role as structural components of the cell membrane, phospholipids serve as precursors for various second messengers such as ArAc, DHA, ceramide, DAG, phosphatidic acid, and lyso-phosphatidic acid. Lipids comprise a large number of chemically distinct molecules arising from combinations of fatty acids with various backbone structures. Overall, mammalian cells may contain 1,000–2,000 lipid species. Oxidative lipid metabolism may be of particular importance for the CNS, as this organ has the highest concentration of lipids next to adipose tissue.

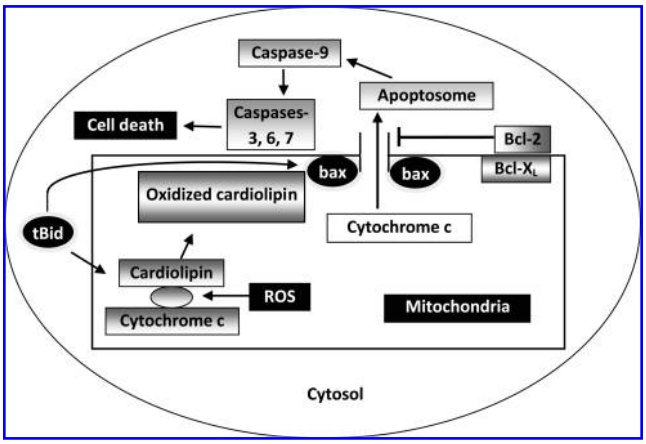


FIG. 2. Cytochrome c release from mitochondria leading to cell death *via* caspase activation (155, 156, 257, 259). Cytochrome c is anchored to the outer surface of the inner mitochondrial membrane by its interaction with cardiolipin. Mitochondrial ROS oxidize cardiolipin by a peroxidase activity of the cardiolipin–cytochrome c complex. Cytochrome c then detaches from the mitochondrial membrane and can be released into the cytosol through pores in the outer mitochondrial membrane. Cardiolipin also serves as a mitochondrial target to the C-terminal cleavage product of Bcl-2, Bid. Bid promotes pore formation in the outer membrane by Bax or Bak, a process that is inhibited by Bcl-2 or Bcl-X_L. In the cytosol, cytochrome c participates in apoptosome formation, resulting in caspase-9 activation and subsequent activation of the executioner caspases-3, -6 and -7.

B. Cholesterol is the precursor for neurosteroid synthesis

Cholesterol is synthesized in the cytosol, beginning with acetyl-CoA. Four stages in cholesterol synthesis involve: (a) condensation of three acetate units to form mevalonate, (b) conversion of mevalonate into activated isoprene, (c) polymerization of six 5-carbon isoprene units (30 carbons) to form squalene, and (d) cyclization of squalene to form cholesterol.

The vast majority of cholesterol in the brain is derived from *de novo* synthesis as virtually no cholesterol is transported from the plasma; cholesterol is synthesized in the neurons, glia (astrocytes), and oligodendrocytes. In the adult brain, the predominant synthesis is by astrocytes; cholesterol is then secreted *via* transport molecules such as ATP binding cassette protein (ABCA1), taken up by lipoprotein receptors on neurons and internalized to the endosome/lysosome (E/L) system (Fig. 3) (30, 361).

Cholesterol is transported to mitochondria by Niemann–Pick C1 (NPC1) protein where it is converted by cytochrome p450 side chain cleavage enzyme to pregnenolone, the rate-limiting intermediate in neurosteroid synthesis (Fig. 3) (29, 60, 146, 361, 362). Pregnenolone is exported from the mitochondria to the cytosol where it is converted to progesterone by 3β-hydroxysteroid dehydrogenase. Progesterone is converted, by successive action of 5α-reductase and 3α-hydroxysteroid dehydrogenase, to 3α,5α-tetrahydroprogesterone or allopregnanolone. Pregnenolone is also converted to 17α-hydroxypregnenolone, the precursor for dehydroepiandrosterone (DHEA), by 17-hydroxylase. These neurosteroids (glucocorticoids, mineralocarticoids, and estradiol) act on nuclear and $NMDA/GABA_A$ receptors to promote neurogenesis and modulate neurotransmission. In the brain, neurosteroids are primarily synthesized in astrocytes; the rate-limiting step is the transport of cholesterol from the outer to the inner mitochondrial membrane by translocator protein-18 (previously known as peripheral-type benzodiazepine receptor (PTBR)) and steroidogenic acute regulatory protein (StAR). Neurosteroids have offered neuroprotection in a wide variety of CNS injuries/disorders (*e.g.*, dementia, stroke, epilepsy, SCI, TBI, AD, PD, and NPC). While both provided benefit, allopregnanolone was more effective than progesterone in reducing infarction after stroke (297). DHEA and allopregnanolone also stimulated neurogenesis (160, 340, 368).

C. Phospholipid metabolism

Phospholipids are primarily metabolized by various phospholipases (types A, C, and D), classified according to substrate specificity and site of hydrolysis of the phospholipid (Fig. 4).

PLA_2s cleave the fatty acid from the *sn*-2 position of the glycerol backbone to release a free fatty acid and form a lysophospholipid. PLA_2 isozymes occur in multiple forms (3, 241) in the mammalian cell and are classified as calcium independent ($iPLA_2$, 84 kDa), and the calcium-dependent cytosolic ($cPLA_2$, 85–110 kDa) and secretory ($sPLA_2$, 14–18 kDa)

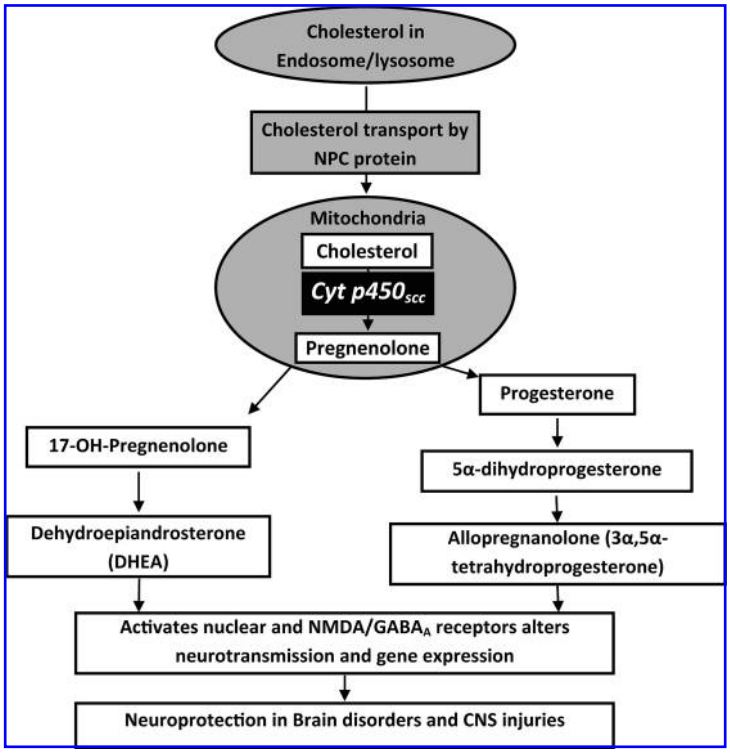


FIG. 3. Synthesis of neurosteroids in the brain and their effects on various brain disorders and injuries. Neurosteroid synthesis in the brain is affected in various brain disorders and injuries and treatment with neurosteroids (pregnenolone, dehydroepiandrosterone, and allopregnanolone) showed positive trend in these brain pathologies. Niemann–Pick C (NPC) protein transports cholesterol from endosome/lysosome system to mitochondria where the neurosteroid synthesis occurs. Pregnenolone is exported from the mitochondria to the cytosol where it is converted to progesterone by 3β-hydroxysteroid dehydrogenase. Progesterone is converted, by successive action of 5α-reductase and 3α-hydroxysteroid dehydrogenase, to 3α,5α-tetrahydroprogesterone or allopregnanolone. Pregnenolone is also converted to 17α-hydroxypregnenolone, the precursor for dehydroepiandrosterone (DHEA), by 17-hydroxylase. DHEA and allopregnanolone may provide beneficial effects based on the following studies: NPC (125), AD, schizophrenia, bipolar disorders, epilepsy (217, 218), PD (381), stroke (297), TBI (82), and SCI (181). Studies showed both DHEA and allopregnanolone stimulated neurogenesis in stroke (218) and increased neuroprogenitor cells in AD models (369).

forms. $cPLA_2$ preferentially cleaves ArAc at the *sn*-2 position of PC and forms lyso-PC, whereas $sPLA_2$s show no fatty acid preference and act on a broader range of phospholipids (1, 9). $iPLA_2$s also are not selective for ArAc and are considered to be involved primarily in membrane remodeling (15, 169). Full activation of $cPLA_2$ requires binding of Ca^{2+} for translocation from cytosol to the phospholipid membrane and phosphorylation of specific serine residues (141). Mitochondrial PLA_2 is a $sPLA_2$ that acts on PC, PE, and cardiolipin (244, 405). Lipoprotein PLA_2 (Lp-PLA_2), a 45 kDa protein, is a group VIIA PLA_2 and is also known as plasma platelet activating factor (PAF) acetylhydrolases (322).

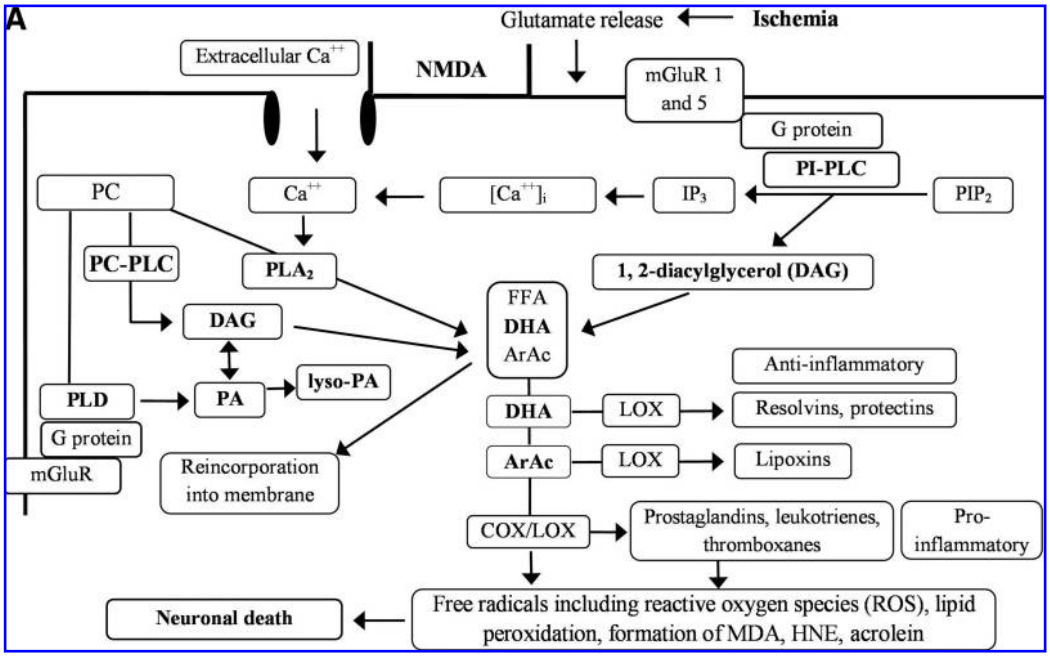
C-type phospholipases hydrolyze between glycerol and the phosphate of the polar head group. Two general types of mammalian enzyme have been identified: PC-PLC hydrolyzes PC to form phosphocholine and DAG. PI-PLC hydrolyzes phosphatidylinositol to form inositol and DAG. When the PI-PLC substrate is phosphatidylinositol 4,5-bisphosphate (PIP_2), inositol 1,4,5-trisphosphate (IP_3) is released. IP_3 is an important second messenger in releasing intracellular calcium from internal stores. Since PC and PI have different fatty acid compositions [PC is highly saturated and composed primarily of palmitic (16:0), stearic (18:0), and oleic (18:1), whereas PI is enriched in stearic (18:0) and arachidonic (20:4) acids], the DAG released by PC-PLC and PI-PLC will have different fatty acid compositions.

PLD catalyzes the hydrolysis of PC to choline and phosphatidic acid (PA). PA may act directly as a signaling molecule and can be further converted to other messenger molecules such as DAG and lyso-PA. PLD catalyzes a unique transphosphatidylation reaction in the presence of a primary alcohol to form a phosphatidylalcohol instead of PA (170). This reaction has been used to measure PLD activity and assess the role of PA in cell signaling, as there are no specific PLD inhibitors. Two mammalian forms (170) have been identified, PLD1 (~125 kDa) and PLD2 (~105 kDa). PLD1 is expressed in neurons and glial cells (170) and PLD2 is expressed in astrocytes (170).

Sphingomyelinases (SMases) are C-type phospholipases with specificity for SM. SMases hydrolyze SM to phosphocholine and ceramide and are broadly categorized based on their pH optimum (alkaline, neutral, and acidic). Alkaline SMase is confined to the intestinal mucosa in many species and in humans is also located in bile and liver (264). Lysosomal acidic SMase (ASMase) is localized primarily in the endolysosomal compartment but can also relocate to the outer leaflet of the plasma membrane (120, 211). A secreted Zn-dependent ASMase (SSMase) is encoded by the same gene as lysosomal ASMase. Both the ~70 kDa lysosomal ASMase and the SSMase can be generated from a single ~75 kDa precursor protein independent of alternate gene splicing (300). Most of the ASMase studies do not discriminate between the relative contributions of SSMase *vs* ASMase (151). Neutral SMase (NSMase) occurs in the endoplasmic reticulum and Golgi apparatus but can also localize to the inner leaflet of the plasma membrane. Once formed, ceramide can be converted by ceramidases to sphingosine, which can then be phosphorylated to sphingosine-1-phosphate (S1P). Ceramide (286) and sphingosine generally exert antiproliferative and proapoptotic effects (267), opposite to those of S1P, which promotes cell growth and inhibits apoptotic signaling (254, 264). Thus, cell fate may be determined by the ratio between ceramide plus sphingosine and S1P levels, rather than their individual levels.

III. ROS and Lipid Peroxidation

Oxidative stress results when production of ROS such as superoxide anion radicals, hydrogen peroxide, and hydroxyl radicals exceeds a biological system's ability to detoxify these



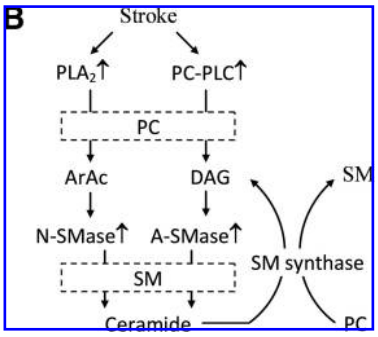


FIG. 4. (**A**) **Lipid metabolism in CNS injury.** Activation of phospholipases (PLA_2, PC-PLC, PI-PLC, and PLD) following cerebral ischemia results in release of lipid second messengers 1,2-diacylglycerol (DAG), phosphatidic acid (PA), lyso-phosphatidic acid (lyso-PA), docosahexaenoic acid (DHA), and arachidonic acid (ArAc). PA and DAG can be readily interconverted by phosphohydrolases and DAG-kinases. ArAc undergoes further metabolism by cyclooxygenases/lipoxygenases (COX/LOX) to generate important signaling and vasoactive eicosanoids. Free radicals are formed during ArAc metabolism by COX/LOX and free radical generation can be induced by eicosanoids. ArAc generates pro-inflammatory prostaglandins, leukotrienes, and thromboxanes, as well as LOX-generated anti-inflammatory lipoxins. Through the LOX pathway, DHA is metabolized to anti-inflammatory resolvins and protectins, including 10,17*S*-docosatriene (Neuroprotectin D1), an endogenous neuroprotectant. (**B**) Glycerophospholipid and sphingolipid relationship. Tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) activate neutral sphingomyelinase (N-SMase) and acidic sphingomyelinase (A-SMase) through stimulation of PLA_2 and PC-PLC and release of ArAc and DAG, respectively. N-SMase and A-SMase hydrolyze sphingomyelin (SM) to liberate ceramide. SM synthase transfers the phosphocholine head-group of PC to ceramide to form SM and DAG.

reactive intermediates (Fig. 5). Measurement of ROS is difficult due to their transient nature. Oxidative stress involves a number of complex ongoing processes, direct and reverse interactions between these processes, and alteration by ROS of a large number of cellular components including proteins, nucleic acids, and lipids (130).

Superoxide anion radicals can combine with reactive nitrogen species (RNS) such as nitric oxide (NO) to generate the strong pro-oxidant peroxynitrite ($ONOO^-$). ROS are produced by a number of cellular oxidative metabolic processes including oxidative phosphorylation by the mitochondrial respiratory chain, xanthine oxidase, NAD(P)H oxidases, monoamine oxidases, and metabolism of ArAc by lipoxygenases (LOX) (3). One of the most abundant sources of ROS, apart from the mitochondrial electron transport chain, is the respiratory burst of activated microglia. This system operates intermittently, but when activated generates large quantities of ROS, particularly superoxide anion radicals, on the microglial outer membrane from which they are released into the surroundings (117). It has been generally accepted that ArAc metabolism by cyclooxygenases (COX) also generates ROS, but recent literature shows that COX-2 does not directly produce ROS but does form carbon-centered radicals on ArAc (178, 320). Most ROS are produced at low levels and any damage they cause to cells is constantly repaired. Low levels of ROS are used in *redox* (reduction/oxidation) cell signaling and may be important in prevention of aging by induction of mitochondrial *hormesis* (*hormesis:* a beneficial response to low

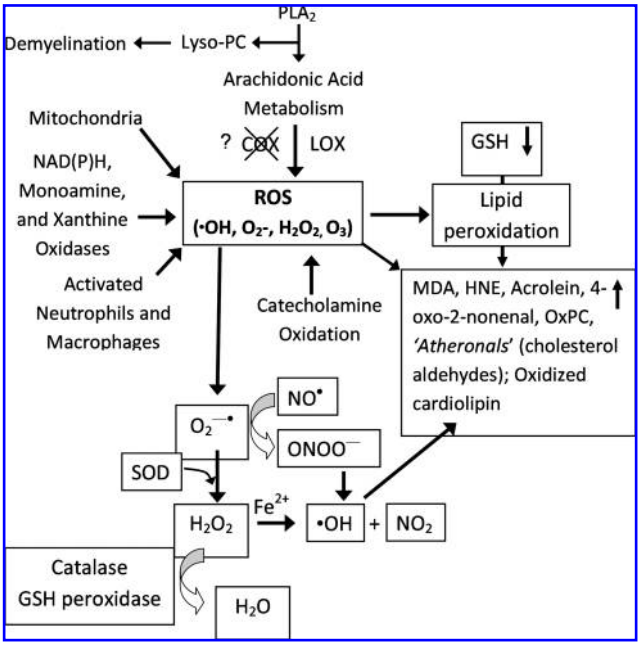


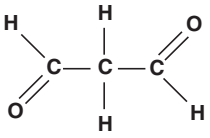
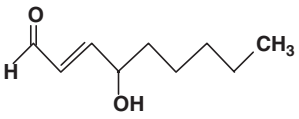
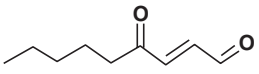
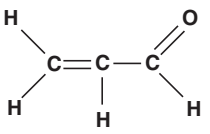
FIG. 5. Different sources of ROS generation leading to various lipid peroxides formation. Recent studies indicate ROS generation from COX-2 is in question (178). Peroxynitrite ($ONOO^-$) is formed by reaction of superoxide anion radical ($O_2^{-\bullet}$) with nitric oxide ($NO^\bullet$). The highly reactive hydroxyl radical •OH is formed either by decomposition of $ONOO^-$ or by reaction of Fe^{2+} with H_2O_2.

dose exposure to toxins) (112, 295). A crucial principle involved with hormesis is that the re-establishment of disrupted homeostasis can result in an adaptive condition that is more beneficial than the prior stage. At higher ROS levels, the beneficial response shifts towards toxicity (112, 295). Generation of ROS is also used by the immune system to destroy invading pathogens (189). Although there are intracellular defenses against ROS, increased production of ROS or loss of antioxidant defenses leads to progressive cell damage and decline in physiological function. Overproduction of these free radicals can damage all components of the cell, including proteins, carbohydrates, nucleic acids, and lipids, leading to progressive decline in physiological function and ultimately cell death.

Several studies have provided evidence that ROS stimulate opening of L-type voltage-sensitive calcium channels (L-VSCC), leading to increased intracellular calcium. Disturbances in cellular calcium homeostasis are involved in the injury and death of neurons that occur as the result of both acute insults such as stroke (390) and chronic neurodegenerative disorders including AD (351).

Beyond the initial damage to membranes, reaction of ROS with double bonds of fatty acids in lipids produces an oxidized phospholipid such as oxidized phosphatidylcholine (OxPC) (154). Scission of the oxidized PUFA results in formation of two aldehyde products: a phospholipid aldehyde such as OxPC, and α,β-unsaturated aldehyde cleavage fragments including malondialdehyde (MDA), 4-hydroxynonenal (HNE), 4-oxo-2-nonenal (ONE) (317), and acrolein (Table 1). Formation of OxPC, a lipid peroxidation product, has been identified in

Table 1. Aldehydes Produced during Oxidative Stress

Structure	*Name*	*Product of*	*Disease*	*Reference*
	Malondialdehyde	Lipid peroxidation	AD, atherosclerosis, stroke	2, 24, 132
	4-Hydroxynonenal	Lipid peroxidation	Cerebral ischemia	3, 353
	4-oxo-2-nonenal	Lipid peroxidation	Triggers p53 pathway; ALS	317
	Acrolein	Lipid peroxidation	Stroke, AD	55, 346
	Atheronals Oxidized PC	Cholesterol ozonolysis	Atherosclerosis Stroke, MS	375 7, 276

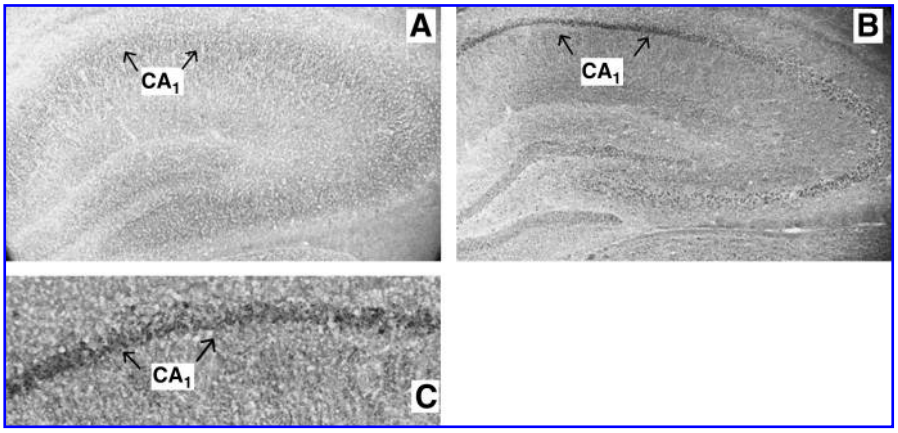


FIG. 6. HNE immunoreactivity in the CA_1 region of the gerbil hippocampus following 10 min of forebrain ischemia and 24 h reperfusion. CA_1 hippocampal region is susceptible for neuronal injury after global ischemia. (**A**) Sham 40×; (**B**) 10 min forebrain ischemia, 24 h reperfusion 40×; (**C**) 10 min forebrain ischemia, 24 h reperfusion 100×. Reproduced with permission from (3).

stroke, as well as in MS (7, 276). Atheronal (cholesterol aldehyde), an ozonolysis product of cholesterol, has been identified in human atherosclerotic arteries (375). These aldehydes covalently bind to proteins through reaction with thiol groups and alter their function. We have previously shown that CA_1 hippocampal neurons were HNE positive by immunohistochemistry after transient cerebral ischemia (3) (Fig. 6). Recently, elevated levels of an acrolein–protein conjugate were demonstrated in plasma of stroke patients (4). Different lipid peroxidation products in various CNS disorders and injuries are listed in Table 1.

F_2-isoprostane is produced by the nonenzymatic free radical peroxidation of ArAc in membrane phospholipids. F_2-isoprostane (isoprostanes containing an F-type prostane ring, a family of 64 prostaglandins) and F_2-like compounds generated *in vivo* are similar in structure to enzymatically generated prostaglandin $F_2\alpha$. There is growing acceptance that measurement of F_2-isoprostanes is a reliable noninvasive approach to assess oxidative stress in patients. Increased levels of isoprostanes have been measured in many conditions that have been associated with excessive generation of free radicals, including brain degeneration and atherosclerotic lesions. Isoprostane analysis has also been used to assess the efficacy of antioxidants *in vivo* and to establish the value of antioxidant administration in clinical trials.

F2-isoprostanes are predominantly derived from ArAc while F4-neuroprostanes, which are the F4-isoprostanes derived from DHA (of which the brain is particularly rich) (249, 288) and other ω-3 fatty acids (eicosapentaenoic, 22:5 and linolenic acid, 18:3) are also markers of neurodegeneration (250) and other neuropathological events linked to oxidative stress and lipid peroxidation. Potential beneficial effects of neuroprostanes as anti-inflammatory mediators has recently been reviewed (243).

The brain is believed to be particularly vulnerable to oxidative stress as it accounts for only 2% of total body weight but consumes 20% of the body's oxygen (325), contains high concentrations of PUFA that are susceptible to lipid peroxidation, is relatively high in redox transition metal ions, yet has relatively low antioxidant capacity compared to other organs (214). Bilirubin, formed by cleavage of heme to biliverdin by heme oxygenase (HO) followed by reduction of biliverdin to bilirubin, is the final product of heme metabolism. Originally considered as a mere by-product of heme degradation, bilirubin is now known to be a potent antioxidant, being able to counteract the cellular damage elicited by ROS as well as RNS and contributes to the overall antioxidant capacity (207, 208, 341) of the brain.

Of all the brain cells, neurons are particularly vulnerable to oxidative insults due to low levels of reduced glutathione (84). Oxidative stress is a component of many neurodegenerative disorders such as PD, AD, MS, and amyotrophic lateral sclerosis (ALS). Oxidative stress may also affect the cell cycle events at the molecular level in many age-related neurodegenerative disorders (171, 409, 410). Increase in ROS production has been identified as an important mechanism by which neuronal plasticity is compromised during aging and after CNS injury. Neuronal and cognitive processes rely on an energy supply to maintain neuronal excitability and synaptic function. Elevated ROS decreases brain derived neurotrophic factor (BDNF)-mediated synaptic plasticity. Mitochondrial activity and BDNF are strongly interconnected through ROS that limit BDNF expression (119). BDNF receptor TrKB, mediates signaling mechanisms coupled with the melanocortin-4 receptor (MC4R), a critical hypothalamic element involved in energy balance. In experimental models, ω3-enriched dietary supplements increased BDNF levels, reduced oxidative damage (protein carbonyls), and enhanced learning and cognitive function after fluid percussion brain injury in rats (118, 385). However, clinical studies indicated a possible increased risk for hemorrhagic strokes, and therapeutic use of ω3-fatty acids in humans needs careful consideration (27).

Thiobarbituric acid reactive substances (TBARS) are a measure of lipid peroxidation. Serum TBARS and BDNF were negatively correlated in bipolar disorder patients, suggesting that alterations in oxidative status may be mechanistically associated with abnormal low levels of BDNF in individuals with bipolar disorder (159). Antidepressants are known to increase serum BDNF in humans (318), and animal model studies demonstrated that mood stabilizers can increase BDNF and regulate TBARS in rodents (100).

IV. Stroke

A. Stroke or "brain attack": A problem of vast clinical importance

Stroke generally refers to a local interruption of blood flow to the brain and is the leading cause of long-term disability, third leading cause of death in United States (399). Approximately 12% of strokes are hemorrhagic (rupture of a cerebral blood vessel), whereas the remaining 88% are ischemic and result from occlusion of a cerebral artery (either thrombolic or

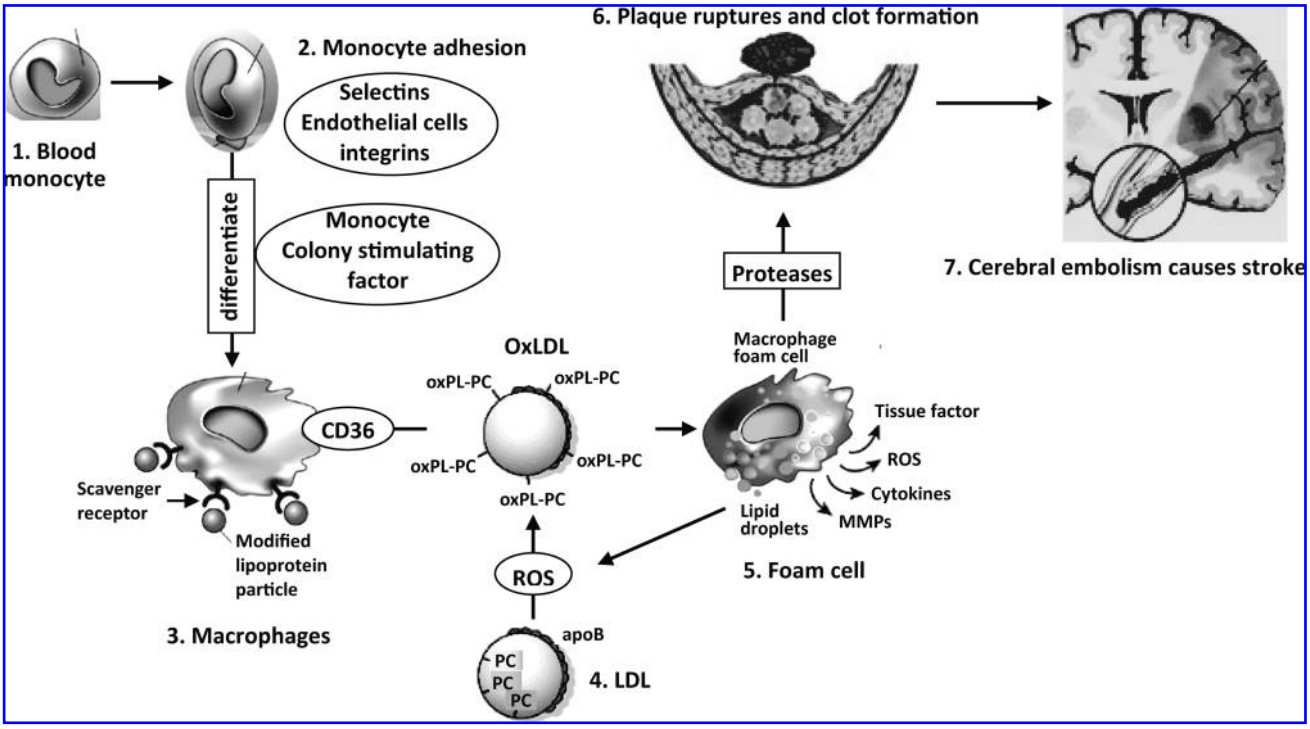


FIG. 7. Atherosclerosis, a major risk factor for ischemic stroke. Under inflammatory conditions (OxLDL, homocysteine, cigarette smoke, shear stress, and infectious agents such as *Chlamydia pneumoniae*) endothelial cells of the artery express adhesion molecules that allow monocytes (**1**) to adhere to endothelia (**2**). Chemoattractants such as monocyte chemoattractants protein-1 (MCP-1) draw the monocytes through the endothelium into the arterial intima. Once resident in the intima, monocytes differentiate into macrophages (**3**) in response to locally produced agents such as monocyte colony stimulating factor. LDL (**4**) under oxidative stress gets oxidized to OxLDL. The macrophages increase expression of scavenging receptors such as CD36, SR-A and SR-B. These scavenger receptors then internalize specifically oxidized LDL (OxLDL, specifically OxPC) particles such that cholesteryl esters accumulate in cytoplasmic droplets, resulting in lipid-loaded macrophages (foam cells, **5**). Foam cells produce ROS that further propagate LDL oxidation, and secrete cytokines and matrix metalloproteinases (MMPs). The MMPs contribute to degradation of the fibrous cap surrounding the plaque, resulting in its rupture and formation of a blood clot (**6**). If the blood clot dislodges from the plaque, arterial blood flow can carry it to the brain, where it lodges in a cerebral artery (embolism) and causes an ischemic stroke (**7**). Reproduced with permission from (8).

embolic). Hypertension is one of the significant risk factors for hemorrhagic stroke; an estimated 17–28% of hemorrhagic strokes among hypertensive patients would have been prevented if they had been on hypertension treatment (383). Blockage of a cerebral artery results in interruption of the blood flow and supply of nutrients, glucose, and oxygen to the brain. The energy needs of the brain are supplied by metabolism of glucose and oxygen for the phosphorylation of ADP to ATP (273, 274).

It has been calculated that *de novo* synthesis of ether phospholipids accounts for 1.4% of total brain ATP consumption and that another 5% is allocated to the phosphatidylinositide cycle. Fatty acid recycling within brain phospholipids and maintenance of membrane acidic phospholipid asymmetries has been estimated to consume 5% and 8% of net brain ATP, respectively. Thus, phospholipid metabolism can consume up to 20% of net brain ATP (273, 274).

Most of the remaining ATP generated in the brain is utilized to maintain intracellular homeostasis and transmembrane ion gradients of sodium, potassium, and calcium. Energy failure results in collapse of ion gradients, and excessive release of neurotransmitters such as dopamine and glutamate (3), ultimately leading to neuronal death and development of infarction. Excess glutamate release and stimulation of its receptors results in activation of phospholipases/sphingomyelinases (3, 10), phospholipid hydrolysis and release of second messengers ArAc and ceramide (3, 7, 11, 227). Ultimately these processes lead to apoptotic or necrotic cell death.

Stroke is characterized by an ischemic core (infarct) surrounded by a "penumbra" (peri-infarct) region that has partial reduction in blood flow due to presence of collateral arteries. The ischemic core is generally considered unsalvageable, whereas the penumbra may be rescued by timely intervention and is a target for the development of therapeutic treatment. Local arterial blockage can be caused by either a thrombus (a clot that forms at the site of the arterial occlusion) or an embolus (a clot that forms peripherally, dislodges into the arterial circulation and is transported to the brain, Fig. 7). Atherosclerosis, discussed in the next section, is the main risk factor for development of these embolisms. Inflammation poses as one of the high risk factors for stroke for its role in the initiation, progression and maturation of atherosclerosis.

B. Atherosclerosis is a risk factor for stroke

Atherosclerosis, a progressive disease of the arteries, is the most common cause of myocardial infarction, stroke, and cardiovascular disease (379). Atherosclerosis is believed to

be predominantly an inflammatory condition produced as a response to injury (89). Atherosclerosis is defined by the accumulation in the arterial intima of mainly low-density lipoprotein (LDL)-derived lipids along with apolipoprotein B-100 (apoB100). LDL is the major carrier of cholesterol in the circulation and is composed of one apoB-100 together with phosphatidylcholine (PC), sphingomyelin (SM), and unesterified cholesterol (500:200:400 molecules, respectively), constituting a surface film surrounding a core of cholesteryl esters and triacylglycerols. Excess free iron generates oxidative stress that hallmarks diseases of aging, including atherosclerosis. A role for iron is also proposed in atherosclerosis, a frequent disorder of aging (17).

The traditional view of atherosclerosis has been simply the deposition and accumulation of cholesterol, other lipids, and cellular debris within the wall of medium to large arteries, resulting in plaque formation and disturbance of blood flow (Fig. 7). The role of cholesterol in atherosclerosis is well established and has been elegantly reviewed (223). It is now believed that a complex endothelial injury and dysfunction induced by a variety of factors such as homocysteine, toxins (smoking), mechanical forces (shear stress), infectious agents (*Chlamydia pneumoniae*), and oxidized LDL results in an inflammatory response that is instrumental in the formation and rupture of plaques, one of the greatest risk factors for ischemic stroke (91, 132).

Two critical events involved in atherogenesis involve accumulation and oxidation of LDL in the arterial intima and recruitment of monocytes to the developing lesion. After diffusion through the endothelial cell junctions into the arterial intima, LDL can be retained through interaction of apoB100 and matrix proteoglycans. LDL accumulates in the arterial intima when its rate of influx exceeds the rate of efflux. While the exact mechanisms governing LDL accumulation remain to be elucidated (246), evidence indicates that LDL uptake and retention are increased at plaque sites, which may involve degradation or binding to cellular and matrix components. Once in the arterial intima, LDL can be oxidized to OxLDL through oxidation of PUFA of LDL lipids, particularly PC of LDL to form OxPC.

A second critical event in atherosclerosis is an inflammatory response that triggers expression of adhesion molecules (selectins and integrins) in the arterial endothelium, stimulating adhesion of monocytes to the endothelium. Monocytes penetrate into the arterial intima, differentiate into macrophages and eventually become foam cells by binding and endocytosing OxLDL through CD36 scavenging receptors (Fig. 7). Since scavenger receptors are not controlled by a sterol-regulated negative feedback loop, macrophages accumulate massive amounts of lipoprotein-derived lipids (265, 404). Studies showed that oxidized phospholipids bearing the PC headgroup as a ligand on OxLDL mediate uptake by macrophage scavenging receptors such as CD36 and scavenger receptor A (SR-A) (45, 339). The macrophage foam cells generate ROS, produce tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1), and matrix metalloproteinase 9 (MMP-9) that promote atherosclerosis, degrade the fibrous cap, and eventually lead to plaque rupture. Exposure of macrophages to HNE, a product formed by ROS-mediated lipid peroxidation, increased expression of class A and CD36 scavenging receptors and a concomitant increase in endocytic uptake of OxLDL (400). Recent studies showed that inflammation is associated with oxidative stress and generation of HNE. *In vivo,* GSH conjugates of HNE, glutathionyl-HNE, formed under oxidative stress are pro-inflammatory (328). Docosanoids are signaling molecules made by oxygenation of 22-carbon essential fatty acids, especially DHA and include some resolvins (nomenclature derived from resolution phase interaction products) and the docosatrienes. Resolvin D1, a lipoxygenase product of DHA (312), effectively counteracted glutathionyl-HNE (GS-HNE) mediated inflammation in human polymorphonuclear leukocytes (328).

Increased levels of TNF-α and IL-1 upregulate expression of adhesion molecules and promote further monocyte recruitment into developing atherosclerotic lesions. Macrophage MMP-9 degrades extracellular matrix components, including the fibrous cap of atheromatous plaques. Rupture of the fibrous cap exposes the blood to the inner components of the plaque, particularly tissue factor released from apoptotic macrophages. Tissue factor binds to activated coagulation factor VII and triggers the coagulation cascade, resulting in formation of a blood clot. Destabilization of this clot results in release of an embolus into the blood stream, which can be transported to the brain, where it can lodge in a cerebral artery and induce an ischemic stroke (Fig. 7) (89, 91, 132, 334).

C. Niemann–Pick C1 (NPC1) and atherosclerosis

Niemann–Pick C1 (NPC1) regulates cellular cholesterol trafficking. *Npc1* deletion in bone marrow-derived cells of chimeric mice led to increased intracellular cholesterol accumulation, cholesterol oxidation, and accelerated atherosclerosis through impaired intracellular cholesterol trafficking and efflux (404). Macrophage expression of NPC1 was critical for generation of an endogenous oxysterol liver X receptor (LXR) ligand (404). Loss of NPC1 leads to faulty suppression of sterol regulatory element binding protein (SREBPs)-dependent gene expression and failure to activate LXR-mediated pathways, resulting in intracellular cholesterol buildup. Persistent activation of SREBP-dependent gene expression results in unregulated cholesterol synthesis and uptake. LXRs regulate cellular cholesterol balance by activation of genes that promote catabolism and elimination of excess free cholesterol and failure to up-regulate LXRs results in defective cholesterol efflux (102, 258). Macrophages regulate lipid homeostasis of plasma lipoproteins (265). NPC1-mediated cholesterol movement contributes to macrophage sterol homeostasis in the arterial vessel and modulates susceptibility to atherosclerosis. NPC proteins normally regulate cholesterol homeostasis by channeling excess LDL cholesterol to intracellular sites of oxysterol synthesis, such as the mitochondrial sterol 27-hydroxylase (CYP27A1), resulting in the generation of oxysterols. In NPC mutants, failure to generate oxysterols may be the key cellular consequence that leads to cholesterol homeostatic defects (258).

D. Nuclear factor erythroid 2-related factor 2 (Nrf2)

Neurodegenerative diseases share various pathological features, such as accumulation of aberrant protein aggregates, microglial activation, macrophage recruitment, and mitochondrial dysfunction. These pathological processes generate ROS that cause oxidative stress and damage to lipids, proteins, and DNA. To maintain proper physiological redox balance, the mammalian cells, including those of the CNS,

have a variety of endogenous antioxidant defenses at the transcriptional level to counteract ROS and oxidative stress. Antioxidant genes that code proteins involved in neutralizing ROS have a common promoter, the antioxidant response element (ARE) (76). ARE-mediated gene activation is regulated by Nrf2, a member of bZIP transcription factors (76). One view of Nrf2 regulation is that, under normal conditions, Kelch ECH associating protein 1 (Keap1), a cytosolic repressor protein that binds to Nrf2, retains it in the cytosol and facilitates its proteasomal degradation. Under oxidative stress conditions, Nrf2 dissociates from Keap1, avoiding proteasomal degradation, and allowing Nrf2 to accumulate and translocate to the nucleus (Fig. 8) (76). Phosphorylation of Nrf2 by various kinases also affects its distribution. PI3K pathway increases the cellular Ca^{2+} necessary for Nrf2 nuclear translocation; Nrf2 activation in combination with activating CCAAT/enhancer binding protein β (C/EBPβ) and PPARγ/retinoid X receptor (RXR) heterodimer contributes to antioxidant phase II gene induction *via* coordinate gene transactivation (158). Nrf2 stability can also be regulated by DJ-1, a cancer and PD-associated protein. DJ-1 prevents the association between Nrf2 and Keap1, thereby preventing Nrf2 ubiquitination and degradation. Nrf2–ARE activation induces the production of a battery of endogenous enzymes, such as superoxide dismutases (SODs), catalase, glutathione peroxidases (GPx's), peroxiredoxins (Prx's), NADPH:quinone oxidoreductases (NQOs), and HO-1. Together, these free radical scavenging enzymes represent a powerful antioxidant defense mechanism (76). Loss of DJ-1/PARK7 leads to NQO1 deficits. This NQO1 deficit is attributed to loss of Nrf2. DJ-1 stabilizes Nrf2 by encouraging dissociation from Keap1, thereby preventing Nrf2 ubiquitination and degradation (67, 206).

E. Nrf2 and atherosclerosis

Given the substantial evidence that oxidative stress and inflammation are key factors in pathogenesis of atherosclerosis, the transcription factor Nrf2 would be expected to have an important role in preventing or reducing atherogenesis by upregulating anti-inflammatory genes. Also, through induction of HO-1, Nrf2 could have anti-atherosclerotic effects by increasing circulating bilirubin levels (individuals with Gilbert's syndrome have high circulating bilirubin levels and lower incidence of cardiovascular disease) (49). Apolipoprotein-E deficient ($ApoE^{-/-}$) mice fed an atherogenic diet is an established model for hyperlipidemia and atherosclerosis. MDA levels in liver were significantly elevated in $ApoE^{-/-}$ $Nrf2^{-/-}$ mice. Surprisingly, $ApoE^{-/-}$ $Nrf2^{-/-}$ mice fed the atherogenic diet exhibited significantly smaller atherosclerotic plaques compared to $ApoE^{-/-}$ controls (339). The decrease in plaque area was associated with reduced uptake of modified LDL by isolated macrophages from $ApoE^{-/-}$ $Nrf2^{-/-}$ mice. Atherosclerotic plaques and macrophages from $ApoE^{-/-}$ $Nrf2^{-/-}$ mice also exhibited decreased expression of scavenger receptor CD36. In contradiction to the reduction in plaques, $ApoE^{-/-}$ $Nrf2^{-/-}$ mice had significantly elevated serum levels of glucose and triglycerides, both of which are associated with promotion of atherosclerosis. Thus, the overall role of Nrf2 is proatherogenic since its antioxidant functions are superseded by upregulation of CD36 receptors and promotion of modified LDL uptake by macrophages (339).

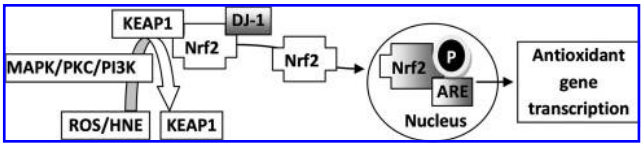


FIG. 8. Nrf2 regulation of antioxidant gene transcription. Under normal conditions, Kelch ECH associating protein 1 (Keap1) binds Nrf2 in the cytoplasm and facilitates its proteasomal degradation. Under oxidative stress conditions, Nrf2 dissociates from Keap1 and translocates to the nucleus. Phosphorylation of Nrf2 by various kinases also affects its distribution. DJ-1 stabilizes Nrf2, probably by promoting its dissociation from Keap1. Nrf2 binds to antioxidant response element (ARE) to initiate antioxidant gene transcription.

F. Lp-PLA$_2$, also known as platelet activating factor (PAF) acetylhydrolase

Lp-PLA$_2$, a 45 kDa protein, is a member of PLA$_2$ family classified as group VIIA PLA$_2$ and is also known as plasma PAF acetylhydrolase (6). This enzyme is found in blood circulation in most animals, and in humans is associated with apoB-100 of LDL and is also found in atherosclerotic plaques (185). In circulation, ~80% of Lp-PLA$_2$ is bound to LDL, whereas the remaining 20% is bound to high density lipoprotein (HDL) and remnant lipoprotein particles (188). Lp-PLA$_2$ is distinct from other phospholipases in that it lacks activity against naturally occurring phospholipids on the cellular membrane, and is the enzyme solely responsible for hydrolysis of oxidized phospholipids in LDL (188). Lp-PLA$_2$ appears to be unique in its high specificity for vascular inflammation and its direct role in causal pathways of plaque inflammation (188). Epidemiological studies have shown that increased levels of Lp-PLA$_2$ were consistently associated with a higher risk for cardiovascular disease (185, 188, 253, 371, 379) independently of conventional risk factors (109). The PLAC test (www.plactest.com) is a FDA-approved blood test measuring Lp-PLA$_2$ to provide supportive information in conjunction with clinical evaluations and other tools to predict patient risk for development of coronary heart disease. Elevated levels of Lp-PLA$_2$ is also associated with dementia (among people aged 55 years and older) independent of cardiovascular and inflammatory factors (358). Lp-PLA$_2$ is produced and secreted by cells of monocyte-macrophage series, T-lymphocytes and mast cells. In addition to PAF acetylhydrolase activity, Lp-PLA$_2$ also hydrolyzes oxidized PC (OxPC) of LDL to generate oxidized fatty acids and lysophosphatidylcholine (lyso-PC) (402). In cultured cells, these two bioactive lipids induce multiple effects consistent with plaque instability (*i.e.*, enhanced inflammation and induction of cell death) (401). Local coronary lyso-PC formation is also associated with endothelial dysfunction and supports the role of this enzyme in vascular inflammation and atherosclerosis in humans. Lp-PLA$_2$ also has an anti-inflammatory function arising from hydrolysis of PAF, which is known to activate platelets, monocytes and macrophages.

One issue of debate has been whether Lp-PLA$_2$ is a causative factor in atherosclerosis or is merely a marker of increased risk of cardiovascular disease (371). Because of its anti-inflammatory activity in degrading PAF, Lp-PLA$_2$ was initially believed to be atheroprotective (345). Identification

of additional substrates for the enzyme led to uncertainty concerning its role in the atherosclerotic process. Despite considerable circumstantial evidence implicating Lp-PLA_2 as a causative factor in unstable plaque formation, definitive proof has been lacking. Mouse models of atherosclerosis have not been useful for resolving these questions as mouse Lp-PLA_2 primarily associates with HDL (108), not with LDL as it does in humans. Also, mice generally do not develop the complex lesions that are prone to rupture (371). A recent study used darapladib, which inhibits Lp-PLA_2 at subnanomolar concentrations, to investigate the role of Lp-PLA_2 in atherosclerotic plaque formation in a diabetic pig model (379). These pigs possess an LDL and HDL profile similar to humans and also develop advanced coronary lesions. Atherosclerosis development was accelerated in this model by induction of diabetes and consumption of a hyperlipidemic diet, which resulted in substantial increases in plasma Lp-PLA_2 activity and arterial lyso-PC content, both of which were significantly attenuated by treatment with darapladib. Darapladib also exerted a general anti-inflammatory effect, reducing the expression of 24 genes associated with macrophage and T lymphocyte functioning. While the drug treatment had only a modest effect on the size of coronary artery lesions, inflammatory cell content and necrotic core area were significantly reduced in the lesions, resulting in fewer lesions with an unstable phenotype (379). These studies demonstrate a crucial role for Lp-PLA_2 in vascular inflammation and development of lesions implicated in pathogenesis of myocardial infarction and stroke.

In a recent multicenter, randomized, double-blind, placebo-controlled study in 959 coronary heart disease (CHD) or CHD-risk equivalent subjects receiving atorvastatin 20 or 80 mg, oral darapladib 40, 80, and 160 mg safely inhibited plasma Lp-PLA_2 activity at 4 and 12 weeks compared with placebo by $\sim$43%, 55%, and 66%, respectively. At 12 weeks, darapladib 160 mg reduced interleukin-6 by 12% and high-sensitivity C-reactive protein by 13.0%, relative to placebo (234). One concern with darapladib treatment is that the agent could have a pro-inflammatory effect by inhibiting the PAF acetylhydrolase activity of Lp-PLA_2, thereby increasing platelet aggregation. However, in this clinical study, Lp-PLA_2 inhibition had no detrimental effect on platelet biomarkers (234). Several studies have indicated that PAF may be degraded by enzymatic pathways independent of Lp-PLA_2. Responsiveness to PAF is not altered in Japanese subjects with a genetic variant of Lp-PLA_2 that results in absence of circulating enzyme. Other clinical trials failed to show measurable benefit of Lp-PLA_2 in conditions believed to be PAF-mediated (234).

Another clinical study (313) compared the effects of 12 months of treatment with darapladib (160 mg daily) in 330 patients with angiographically documented coronary disease. Lp-PLA_2 activity was inhibited by 59% with darapladib. After 12 months, there were no significant differences between darapladib and placebo groups in plaque deformability or plasma high-sensitivity C-reactive protein. In the placebo group, there was a significant increase in necrotic core volume, a key determinant of plaque vulnerability, and darapladib halted this increase. These intraplaque compositional changes occurred without a significant treatment difference in total atheroma volume (313). These findings suggest that Lp-PLA_2 inhibition may represent a novel therapeutic approach.

G. Atherosclerosis and group IIA secretory PLA_2 (inflammatory PLA_2)

Group IIA phospholipase A_2 (secretory PLA_2 ($sPLA_2$), also known as inflammatory PLA_2) has been found in human atherosclerotic lesions. $sPLA_2$ IIA is implicated in chronic inflammatory conditions such as arthritis and may also contribute to atherosclerosis. Elevated $sPLA_2$-IIA concentration and activity in healthy people were predictive of future risk of coronary heart disease. In acute myocardial infarction patients, increased levels of $sPLA_2$-IIA were predictive of increased mortality (290, 291). $sPLA_2$ IIA is a pro-atherogenic factor and has been suggested to regulate collagen deposition in the plaque and fibrotic cap development (113). $sPLA_2$ is one of the enzymes responsible for the release of lyso-PC *via* its catalytic action and these two play a crucial role in the development of atherosclerosis (174). Compositional changes of apolipoprotein B in $sPLA_2$-modified LDL mediate increased binding to human aortic proteoglycans. The anchoring of $sPLA_2$ to proteoglycans results in further remodeling of intimal LDL particles with eventual formation of LDL aggregates. These aggregates are rapidly cleared by tissue macrophages, leading to foam cell formation (291). Noncatalytic (nonenzymatic) atherogenic effects of $sPLA_2$ II are thought to involve binding to a muscular-type (M-type) $sPLA_2$ receptor.

A recent phase-II clinical trial examined dose-response effects of the $sPLA_2$ inhibitor A-002 (1-H-indole-3-glyoxamide; inhibition $sPLA_2$-IIa $\geq$ $sPLA_2$-X $>$ $sPLA_2$-V) in 393 patients with stable coronary heart disease (291). Patients received 50, 100, 250, or 500 mg twice daily for 8 weeks. A-002 treatment resulted in progressive dose-dependent decreases in serum $sPLA_2$ concentration to nearly an order of magnitude less than baseline. Although the mechanism needs further investigation, it was postulated that A-002 might reduce $sPLA_2$-IIA concentrations by interfering with the autocrine pathway in which $sPLA_2$ augments its own production. A-002 attenuated measures of vascular (oxidized LDL concentration) and general (C-reactive protein) inflammation, and LDL cholesterol concentrations, mainly by reducing small dense LDL particles. While these studies indicate that A-002 might be an effective anti-atherosclerotic agent, further clinical studies are needed to determine whether the agent will significantly decrease cardiovascular events in the clinical setting. Table 2 outlines the contributions of different forms of PLA_2 in various CNS pathologies.

H. Sphingomyelinases (SMase) and ceramide: More culprits in atherosclerosis

Endothelial cells, which cover the atherosclerotic lesion, secrete SSMase and secretion is enhanced by pro-inflammatory cytokines. SSMase hydrolyzes SM on the surface of atherogenic lipoprotein particles, even at neutral pH (264). LDL also possesses SMase activity, which may be intrinsic to apoB-100 (168). LDL of atherosclerotic lesions is highly enriched in both SM and ceramide compared to plasma LDL (40). The increase in lipoprotein ceramide promotes fusion and subendothelial aggregation of LDL, increasing their affinity for arterial wall proteoglycans and leading to foam cell formation, critical steps in the initiation of atherosclerosis (168, 264). Studies using transgenic mice have shown that ASMase deficiency reduced both arterial trapping of atherogenic lipoproteins and lesion development (79).

Table 2. Role of PLA_2 in CNS Pathologies (7)

CNS pathology	*Role of PLA_2*
Alzheimer's disease (AD)	Upregulation of PLA_2, increased lipid peroxidation (93, 94, 380). Expression of $sPLA_2$ IIA (238) and $cPLA_2$ (333) increased in AD brains. Aβ induced mitochondrial dysfunction through $iPLA_2$ and $cPLA_2$. Aβ induced $cPLA_2$ phosphorylation *via* NADPH oxidase and ROS production (28, 316, 408). $cPLA_2$ reduction ameliorates cognitive deficits in a mouse model (294).
Dementia	Lp-PLA_2 has been associated with risk of dementia (358).
Atherosclerosis	$sPLA_2$ inhibition reduces LDL cholesterol and both $sPLA_2$ and Lp-PLA_2 inhibitors mitigate inflammatory processes (290, 371, 379).
Parkinson's diseases (PD)	$cPLA_2$ knockout mice showed protection against MPTP toxicity (94, 172).
Multiple sclerosis-Experimental autoimmune encephalomyelitis (MS-EAE)	$cPLA_2$ is highly expressed in EAE (94). $cPLA_2$-deficient mice are resistant to EAE (157, 215, 216). $sPLA_2$ activity increased and inhibition by CHEC-9 blocks inflammation (74).
Neurodegeneration with brain iron accumulation (NBIA)	INAD (NBIA type II) and the related Karak syndrome are caused by mutations in the gene encoding PLA2G6 ($iPLA_2$) (203, 236).
Spinal cord injury (SCI)	$cPLA_2$ expression, PLA_2 activity were increased (196); Mepacrine reduced PLA_2-induced neuronal death. The studies did not directly assess the role of endogenous PLA_2 in neuronal injury after SCI. Major part of the studies conducted injecting PLA_2 or mellitin to spinal cord of normal rats and showed increases in TNF-α, IL-1β and HNE.
Transient focal cerebral ischemia (Stroke)	Activation of PLA_2 and increased $sPLA_2$ expression (3, 11, 193). $cPLA_2$ knockout mice showed protection (3, 43). CDP-choline attenuated $sPLA_2$ (11). PLA_2 inhibitors, quinacrine and indoxam, reduced the infarction size (92, 94, 389). Transgenic mice overexpressing Lp-PLA_2/PAF-acetylhydrolase had smaller infarcts and fewer neurological deficits (352).
Wallerian degeneration	PLA_2 plays an important role in myelin breakdown and phagocytosis. PLA_2 expressed during the early stage of Wallerian degeneration hydrolyzes PC in myelin to LPC and ArAc (75). LPC can aggravate the inflammatory responses that can further upregulate $cPLA_2$ in a positive feedback manner.

Apoptosis of vascular smooth muscle cells is a critical event in the rupture of atherosclerotic plaques, leading to thrombosis (formation of a blood clot within the artery). In cultured vascular smooth muscle cells, apolipoprotein C-1 (apoC-1)-enriched HDLs stimulate NSMase, triggering an apoptotic response. Also, oxidized phospholipids that are found in atherosclerotic lesions may promote vascular smooth muscle cell death via ASMase activation. *In vivo* high-resolution MRI techniques established the co-localization of apoC-1, ceramide, caspase-1 and -3 in regions of plaque rupture, supporting the relevance of the *in vitro* findings (264).

Ceramide is also formed through a *de novo* synthesis pathway. The first and rate-limiting step in ceramide biosynthesis is the condensation of palmitoyl CoA with serine catalyzed by serine palmitoyltransferase (131). Studies in hyperlipidemic apoE-deficient mice have demonstrated that ceramide formed by *de novo* synthesis contributes to atherosclerotic plaque formation. Treatment of apoE-deficient mice with myriocin, a potent inhibitor of serine palmitoyltransferase, significantly lowered plasma sphingolipids and atherogenic plasma lipids, leading to the regression of pre-existing atherosclerotic lesions and to the formation of a stable plaque phenotype (263).

Elevated plasma homocysteine levels are an established risk factor for atherosclerotic coronary heart disease and cerebrovascular disease (343). Homocysteine was shown to increase ceramide levels in rat mesangial cells; the increase was not due to activation of SMase, but to stimulation of *de novo* ceramide synthesis (394).

I. Therapeutic potential

A number of phospholipases, including Lp-PLA_2, $sPLA_2$, NSMase and ASMase, have been implicated in the initiation and progression of atherosclerosis and represent potential therapeutic targets. Because of this multifactorial involvement, drugs directed to only one of these phospholipases may be of limited benefit. Since three forms of NSMase have been identified, further studies are needed to elucidate the contribution of each form to atherosclerosis, and whether pharmacological inhibitors can be developed with the appropriate specificity. Because of its secretion by endothelial cells, secretory SMase may be a preferred target over the lysosomal ASMase, which has an important housekeeping role in lysosomes (264).

J. Cytokines and stroke

Stroke initiates the immune response, activating inflammatory cells including microglia/ macrophages and generating ROS. ROS can further stimulate release of cytokines that cause upregulation of adhesion molecules, mobilization and activation of leukocytes, platelets, and endothelium (370). These activated inflammatory cells also release cytokines, MMPs, nitric oxide, and additional ROS in a feedback fashion (370). ArAc metabolites, synthesized by and liberated from astrocytes, microglial cells, and macrophages, are intimately involved in the inflammatory process by enhancing vascular permeability and modulating inflammatory cell activities and ROS generation. Particularly, cyclooxygenases (COX-1 and

COX-2, prostaglandin endoperoxide synthases) are key rate-limiting enzymes in the conversion of ArAc to prostaglandins and other lipid mediators. It has been suggested, due to its localization in microglia, that COX-1 might be the major player in neuroinflammation, whereas COX-2 (localized to pyramidal neurons), might have a critical role where these neurons are under attack (64). COX-2 deletion or treatment with COX-2 inhibitor celecoxib in mice challenged with lipopolysaccharide (LPS), increased the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and NADPH oxidase subunits (14). The role of ROS in activation of various signaling pathways such as p38, JNK, p53, ERK1/2, Akt, NF-κB (72), MMPs (195), and stroke injury (3, 212) have been recently reviewed.

There are substantial data from both animal models and clinical studies that cytokines, including TNF-α, IL-1 and IL-6, are upregulated after stroke. Although the roles of cytokines in stroke pathology remain controversial, the majority of studies support their deleterious effects, at least in the early phase of stroke injury. Whether cytokines mediate pro-survival or pro-apoptotic signaling appears to depend on their concentration, the target cell, the activating signal, and the timing and sequence of action (6). A number of studies have demonstrated that TNF-α and IL-1 modulate phospholipid and sphingolipid metabolism by upregulating phospholipases and SMases and downregulating enzymes of phospholipid/sphingolipid synthesis, although most of these studies were conducted in cell lines not of CNS origin. The release of ArAc during ischemia may be one of the initial events that upregulate cytokine expression. Ceramide released by SMases triggers the MAP kinase cascade and can upregulate cytokine expression through activation of NF-κB. Thus phospholipid metabolism and cytokine expression (the inflammatory response) may function through a feedback mechanism. The integration of cytokine biology and lipid metabolism in stroke is less explored and was recently reviewed (6). Inflammatory response after stroke suggests that cytokines (TNF-α, IL-1α/β, and IL-6) affect the phospholipid metabolism and subsequent production of eicosanoids, ceramide, and ROS that may potentiate stroke injury. PC and SM are sources for lipid messengers. SM synthase serves as a bridge between metabolism of glycerolipids and sphingolipids. TNF-α and IL-1α/β can induce phospholipases and sphingomyelinases, and concomitantly proteolyse PC and SM synthesizing enzymes. Together, these alterations contribute to loss of PC and SM after stroke that can be attenuated by inhibiting TNF-α or IL-1α/β signaling.

A systemic inflammatory response involving upregulation of TNF-α and IL-1 is believed to be instrumental in the formation and destabilization of plaques, one of the risk factors for ischemic stroke (91, 132). There is considerable clinical data indicating that this systemic inflammation is associated with unfavorable outcome in stroke patients (224). However, this inter-relationship of systemic inflammation with stroke pathology has not been well studied.

K. Antioxidants and stroke

EPC-K1, a phosphodiester of vitamin C and E that inhibits PLA_2 activity and lipid peroxidation, reduced spatial learning deficits following 20 min transient global ischemia (4-vessel occlusion) in male Wistar rats. In another study, EPC-K1 significantly decreased both cerebral lipid peroxidation and infarct in transient focal cerebral ischemia, indicating the contribution of lipid peroxidation to ischemic brain injury (3).

Tocotrienols, formerly known as zeta, or eta-tocopherols, are similar to tocopherols except that they have an isoprenoid tail with three unsaturation points instead of a saturated phytyl tail. Tocotrienols possess powerful antioxidant, anticancer, and cholesterol-lowering properties. At nanomolar concentration, α-tocotrienol, but not α-tocopherol, completely protected neurons by an antioxidant-independent mechanism. α-Tocotrienol (a member of the family of vitamin E compounds) (299), but not natural vitamin E, offered protection in stroke models by suppressing glutamate-induced cell death mediated through early activation of c-Src kinase and 12-lipoxygenase pathways (164–166, 309, 310). Micromolar, but not nanomolar, α-tocotrienol functioned as an antioxidant in linoleic acid induced oxidative stress and cell death in mouse hippocampal HT4 neural cells. Both homocysteic acid and linoleic acid caused neurotoxicity by increasing the ratio of oxidixized to reduced glutathione (GSSG/GSH), raising intracellular calcium and compromising mitochondrial membrane potential (164).

The three peroxisome-proliferator-activated receptor (PPAR) subtypes (α, β/δ, and γ) are nuclear receptors that are recently recognized to play an important role in CNS disorders and injuries (139). Recent studies using neuron-specific PPARγ null mice reinforce the neuroprotective function of PPARγ and the growing body of evidence recognizing activation of PPARγ as a possible target for neuroprotection in CNS disorders and injuries where oxidative stress is a major player (70, 406). These studies also indicate that PPARγ in neurons is not essential for normal neuronal well being, but it plays an important role in protecting neurons from damage by ischemic and oxidative injury.

As far as neurosteroids are concerned, while both provided benefit, allopregnanolone was more effective than progesterone in reducing infarction after stroke (297). DHEA and allopregnanolone also stimulated neurogenesis (160, 340, 368).

L. Stroke clinical trials

Presently, tissue plasminogen activator (tPA) is the only FDA approved drug for stroke treatment, but has limited utility due to its narrow 3 h treatment window. The failure of stroke clinical trials of NXY-059 (a nitrone-based spin trapping agent) emphasized the dire need for new treatment strategies (8, 96, 106). More than 1,026 neuroprotective agents have been tested in animals; of these, 114 have gone to clinical trials, but none were successful in stroke treatment other than tPA (251). Most of the time the failures have been attributed to delayed treatment that ranged beyond 3 h to 1 day (106). Certainly one of the challenges facing stroke treatment is that only a small percentage of patients can be transported to the hospital and begin treatment within the first 3 h. Though delayed treatment is preferable, the concept that *'time is brain'* wins (the sooner the treatment, the better will be the recovery) since ~2 million neurons die every minute after stroke (83, 296). Virtually all previous stroke clinical trials have been based on monotherapy. Combination treatments (neuroprotectant plus thrombolytic) such as magnesium during the first hour after ischemic stroke to protect brain tissue followed by tPA treatment to restore blood flow are underway.

Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, MCI-186) is a novel potent free radical scavenger. It was first reported to have a beneficial effect in animal models of stroke in the late 1980s. In June 2001, it was approved by the Japanese regulatory authorities as the first free radical scavenger for clinical use in the management of acute ischemic stroke (122). Now it is widely used in China. Numerous experimental studies have showed that edaravone has neuroprotective properties due to its antioxidant effects; the reported mechanisms are: (a) quenching hydroxyl radical and inhibiting hydroxyl radical-dependent and hydroxyl radical-independent lipid peroxidation (391); (b) inhibiting both water-soluble and lipid-soluble peroxyl radical-induced peroxidation systems; and (c) inhibiting both nonenzymatic lipid peroxidation and lipoxygenase pathways and has potent antioxidant effects against ischemia or reperfusion-induced vascular endothelial cell injury, delayed neuronal death, brain edema, and concomitant neurological deficits. Combination of edaravone along with hyperbaric oxygen was investigated in stroke patients and found to be effective for treatment of embolic stroke in a pilot clinical trial (144).

Lazaroids (21-aminosteroids) are steroid analogs that specifically inhibit lipid peroxidation without glucocorticoid/mineralocorticoid activity that eliminates the coritocosteroid complications (162). Tirilazad mesylate (a nonglucocorticoid 21-aminosteroid/lazaroid) inhibits lipid peroxidation. Tirilazad offered benefits in experimental models of TBI, SCI, subarachnoid hemorrhage (SAH), and cerebral ischemia models. However, in acute stroke clinical trials, tirilazad increased the death and disability (71).

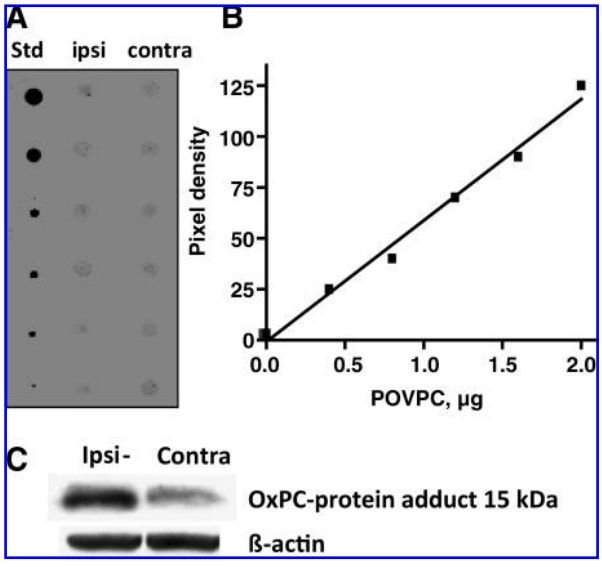


FIG. 9. Evidence for oxidized phosphatidylcholine (OxPC) formation after stroke (7). (**A**) Dot blot of POVPC standard (1-palmitoyl-2-(5′-oxo)-valeryl-sn-glycero-3-phosphorylcholine) and lipid extracts from contra- and ipsi-cortex after 1 h MCAO and 24 h reperfusion using EO6 antibodies (Courtesy: Dr Witztum, Univ of California; San Diego). Free OxPC in brain extracts was very low. (**B**) Standard curve generated from pixel density of POVPC. (**C**) Western blot of OxPC-protein adduct in ipsi-cortex after tMCAO ($n = 3$).

M. Oxidized PC (OxPC) is an inflammatory marker

We have previously shown that PC loss, either due to activation of phospholipases or inhibition of its synthesis *via* CTP:phosphocholine cytidylyltransferase (CCT), may be a significant factor contributing to stroke injury that was attenuated by treatment with CDP-choline (a phase III clinical trial drug for stroke treatment) (2–4, 11). Another contributing factor for PC loss could be conversion to OxPC. OxPC itself also changes the membrane properties, resulting in alterations in ion transport and membrane protein function (154). The presence of OxPC on the apoptotic cell surface has been characterized by EO6 monoclonal antibodies that exclusively bind to OxPC (276). In addition to OxPC, EO6 antibodies also recognize OxPC bound to lysine residues of proteins. OxPC on apoptotic cells may enhance pro-inflammatory signals and also serve as a marker of inflammation and apoptosis (48, 59, 154). The presence of OxPC has been demonstrated in MS brain using EO6 monoclonal antibodies (276). Formation of OxPC species were also shown after permanent focal ischemia in mice (105). Relative generation of OxPC and OxPC-modified proteins in the ischemic hemisphere *vs* contralateral region was assessed by dot blots and Western blot after transient focal cerebral ischemia and 1 day reperfusion ($n = 3$). Our studies show that there was no detectable amount of OxPC (evidenced by dot blot). However there was an increase in OxPC-modified protein (~15 kDa) in the ischemic cortex compared to contralateral cortex. Presently the identity of the modified 15-kDa protein is unknown (7, 276) (Fig. 9). Time course of changes related to cytokines, lipid metabolism, and oxidative stress after stroke has been presented (Fig. 10).

Using an antibody to HNE-modified proteins, no HNE immunoreactivity was detected at 1 h, but was detectable in neurons within the infarcted zone at 3 h and in the boundary between infarcted and noninfarcted zones over 6–48 h of reperfusion following 3 h of focal cerebral ischemia in rat (350). Expression of Bcl-2 attenuates ischemic injury. In transient forebrain ischemia, the hippocampal CA_1 neurons destined to die cease making Bcl-2 protein (61), and it is conceivable that increased levels of HNE-modified proteins are due, at least in part, to lack of expression of Bcl-2, resulting in deficient ability to trigger various antioxidant defense systems (63, 247, 277).

The proteasome is a large 700 kDa complex that performs the majority of protein degradation and is responsible for removal of most oxidized, aggregated, or damaged proteins. Activity of the proteasome complex decreased following transient focal cerebral ischemia in mice, without a corresponding decrease in protein expression of the proteasome subunits. Levels of HNE-modified proteasome complex subunits increased as early as 1 h reperfusion, suggesting that the loss of proteasome activity was due to the modification by HNE. GSH-Px-deficient mice showed further increased levels of HNE-modified proteasome subunits (163).

In permanent focal cerebral ischemia in rat, HNE immunoreactivity increased in the ipsilateral hemisphere 4 h after induction of MCAO. HNE immunoreactivity extended beyond the area of ischemic damage, suggesting that HNE-modified proteins accumulated in tissue prior to development of infarction (3). In another study, MDA and conjugated-diene levels were significantly elevated in ipsilateral compared to

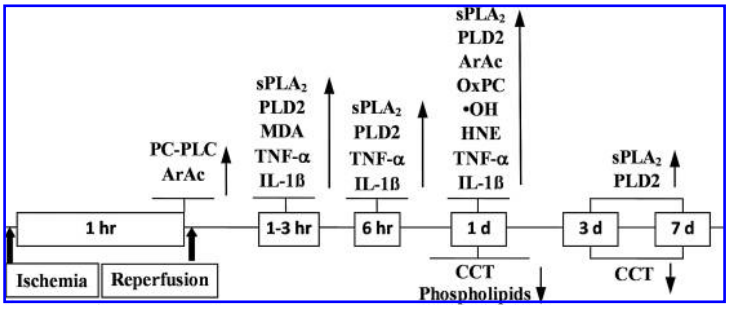


FIG. 10. Time course of changes related to cytokines, lipid metabolism, and oxidative stress after transient brain ischemia. ↑, increase; ↓, decrease, compared to control. ArAc, arachidonic acid; CCT, cytidine triphosphate:phosphocholine cytidylyltransferase; HNE, 4-hydroxynonenal; IL-1β, interleukin-1β; MDA, malondialdehyde; •OH, hydroxyl radical; PLA_2, phospholipase A_2; PC-PLC, PC-phospholipase C; PLD2, phospholipase D2; PLA_2 enzyme activity, $sPLA_2$ mRNA and protein expression, PC-PLC activity and PLD2 protein expression were increased after stroke. CCT catalyzes the rate-limiting step in the biosynthesis of PC. CCT activity and protein expression decreased following stroke. Activation of phospholipases and loss of CCT collectively resulted in loss of PC.

contralateral cortex following 1 h of permanent MCAO (3). To date, studies on aldehyde products in ischemic brain have focused on HNE and have shown increased lipid peroxidation in several models of cerebral ischemia. Recent studies suggested acrolein as a novel biochemical marker for stroke diagnosis; however acrolein formation was attributed to the polyamine oxidase pathway (12, 58, 346, 397, 398).

N. Inflammation and resolution

A critical aspect of the inflammatory response is the ability to stop the inflammation, referred to as the resolution phase, an active process involving expression of anti-inflammatory agents (311, 312). Activation of PLA_2s release ArAc, eicosapentaenoic acid, and DHA. ArAc is metabolized to eicosanoids (prostaglandins, leukotrienes, and thromboxanes) through the COX/LOX pathways, a major pathway mediating inflammation, but is also metabolized to anti-inflammatory lipoxins through the LOX pathway. Chemical mediators such as aspirin can acetylate COX-2; prostaglandin synthesis is inhibited and metabolism is shifted by acetylated COX-2/LOX pathway to generate pro-resolution lipoxins. Eicosapentaenoic acid and DHA, ω-3 fatty acids, are metabolized to resolvins (328) and protectins such as neuroprotectin D1 (NPD1) that have important roles in resolution of inflammation (311).

V. Traumatic Brain Injury (TBI)

TBI is associated with significant neuropsychological deficits, primarily in the domains of attention, executive functioning, and memory. In TBI, the initial traumatic event is shearing, laceration, and/or contusion of brain tissue resulting from a physical impact. Secondary injury after the initial trauma results from ischemia, alterations in ion and neuromodular levels, oxidative stress caused by ROS, edema, and axonal swelling (287).

The inflammatory cascade that follows TBI may lead to secondary cell death and can impede recovery of function (363). Complement factors and their convertases are increased in glia after brain injury and lead to the production of inflammatory products that kill vulnerable neurons. Complement C3 is a key component in the activation of the complement system. The C3 precursor is 185kD protein that is cleaved into α (120 kD) and β chain (75 kD) linked by a disulfide bond. This mature C3 is further cleaved by C3 convertase to release C3a and C3b. C3a, one of the anaphylatoxins of the complement system, is an immune cell activator and a promoter of inflammatory cytokines in human disease processes such as cerebral ischemia (233) and AD (42, 90). C3b participates in cellular adherence and enhances phagocytosis in addition to its crucial role in forming cellular intermediates that perpetuate the complement activation process. Such a self-promoting inflammatory system can be detrimental if left unchecked. Disrupting the activity of convertase could interrupt this injury cascade. CD55, a single-chain type 1 cell surface protein, is a potent inhibitor of the complement convertases which are activators of the inflammatory cascade.

The neurosteroid progesterone and its metabolite allopregnanolone reduced the expression of inflammatory cytokines in the acute stages of brain injury. Both progesterone and allopregnanolone treatments enhanced the production of CD55 following contusion injuries of the cerebral cortex in rats (363). DHEA and allopregnanolone reduced cell death, astrogliosis, and functional deficits in rats after TBI (82). Progesterone and allopregnanolone have been shown to reduce edema, inflammation, and lipid peroxidation in several models of brain injury (331). Progesterone treatment significantly reduced 8-isoprostane (8-epi $PGF_{2\alpha}$), a vasoconstrictive free radical-generated prostaglandin, 1–2 days after cortical contusion. Progesterone and allopregnanolone may control the expression of inflammatory mediators through a CD55-regulated mechanism. By breaking the chain of injury-induced inflammation at the point of amplification, molecular upregulation of CD55 by progesterone and allopregnanolone helps to explain how these two neurosteroids reduce the neuropathology related to inflammation such as blood brain barrier dysfunction, cerebral edema, and apoptosis associated with TBI (363).

These neurosteroids were also shown to affect coagulating factors. After TBI, progesterone treatment generally maintained procoagulant (thrombin, fibrinogen, and coagulation factor XIII), whereas allopregnanolone increased expression of anticoagulant tPA. Progesterone significantly increased the ratio of tPA bound to neuroserpin, a serine protease inhibitor that can reduce the activity of tPA (364). These findings suggest that in TBI, where blood loss may exacerbate injury, treatment with progesterone may be preferable, whereas allopregnanolone may be more appropriate for thrombotic stroke, where a reduction in coagulation would be more beneficial (364). This also suggests stimulating local synthesis of progesterone may offer benefits after TBI or CNS lesions. Translocator protein 18kDa (formerly known as peripheral type benzodiazepine receptor, PTBR) is an intramitochondrial cholesterol transporter that may stimulate neurosteroid synthesis and promote neuroprotection/neuroregeneration (305).

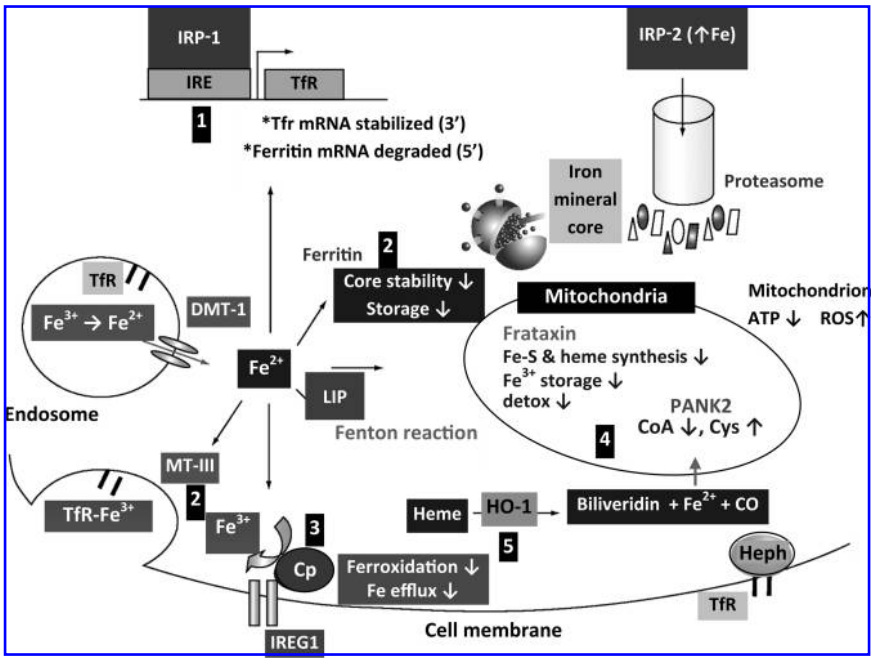


FIG. 11. Intracellular fate of ferrous iron. The uptake of iron in the ferric form (Fe^{3+}) is mediated by the transferrin receptor (TfR) and the reductase present in acidified endosomes converts Fe^{3+} to Fe^{2+}(ferrous) before transport into the cytosolic space by divalent metal transporter-1 (DMT-1). The resultant Fe^{2+} within the labile iron pool (LIP) either: (**1**) activates the regulatory iron regulatory protein/iron regulatory element (IRP/IRE) system, (**2**) gets sequestered by chaperones or storage proteins [such as ferritin, metallothioneins (MTs), and frataxin], (**3**) re-oxidizes into Fe^{3+} and effluxes out of the cell via ceruloplasmin (Cp) and IREG1, or (**4**) participates in iron-catalyzed reactions to generate reactive species [*e.g.*, pantothenate kinase 2 (PANK2)-deficient oxidation of cysteine residues]. (**5**) Additionally, Fe^{2+} can be liberated from free heme groups to contribute to the LIP. Heph, hephaestin, a membrane-bound protein homolog of Cp. Reproduced with permission from ref. 187.

Corticosteroids have been proposed as therapies to reduce secondary injuries following TBI. Corticosteroids inhibit the PLA_2/COX/LOX pathways, thus limiting ArAc release and metabolism, downregulating pro-inflammatory cytokines and attenuating inflammatory responses. However, large scale clinical trials of corticosteroids and lazaroids (21-aminosteroids) for treatment of TBI have either failed to demonstrate efficacy or found increased risk of mortality (287).

A. TBI and lipid peroxidation

The Nrf2-ARE pathway is activated after TBI and may be an important regulator in reducing oxidative stress, inflammatory damage, and accumulation of toxic metabolites (392). One day after TBI, the nuclear Nrf2 protein level and the mRNA levels of both HO-1 and NQO1 were significantly upregulated; immunohistochemical studies showed that Nrf2 and HO-1 were localized in neurons as well as in glial cells (392). Increasing evidence has demonstrated the protective role of Nrf2-regulated gene products in CNS diseases. HO-1 activity reduced ROS production by generating biliverdin, which could be further reduced by biliverdin reductase to the potent antioxidant bilirubin (Fig. 11) (392). HO-1-derived iron very frequently reacts with ROS, triggering lipid peroxidation and promoting neuroinflammation. Thus HO-1 has a Janus nature as both a pro-oxidant (by releasing Fe^{2+} for Fenton reaction) or antioxidant (increasing the bilirubin content) (207, 208). However, it has been reported that HO-1-dependent release of iron ions stimulates the expression of the iron-sequestering protein ferritin, which could prevent the Fenton reaction conversion of H_2O_2 to hydroxyl radicals. Importantly, HO-1 and ferritin are co-regulated by Nrf2. NQO1 catalyzes the two-electron reduction and detoxification of quinones and their derivatives, and is considered to protect cells against the adverse effects of quinones and related compounds (392).

Gender differences have been reported in the pathophysiology and outcome of acute neurological injury (26). Greater neuroprotection in females versus males may be due, in part, to sex hormone-mediated antioxidant mechanisms. Progesterone administration decreases brain levels of F_2-isoprostane, a marker of lipid peroxidation, after experimental TBI in male rats, and estrogen is neuroprotective in experimental neurological injury (26). CSF levels of F_2-isoprostane were measured on day 1, 2, and 3 after severe TBI in adults (26). F_2-isoprostane was approximately twofold higher in males (145.8 ± 39.6 pg/mL) than females at day 1. CSF F_2-isoprostane was also associated with hypoxemia (Insufficient oxygenation of arterial blood). Patients treated with hypothermia were cooled to 32°–33° C (within ~6 h) for either 24 or 48 h and then rewarmed. Hypothermia tended to decrease F_2-isoprostane levels only in males on day 1 after TBI. These studies showed that lipid peroxidation occurs after severe TBI in adults and is more prominent in males *vs* females (26). A word of caution should be mentioned: in these studies Bayir *et al.* (26) used a commercial enzyme immunoassay (EIA) kit from Cayman Chemicals (Ann Arbor, MI). Proudfoot *et al.* (271) and Bessard *et al.* (37) compared the isoprostane measurements using gas-chromatography mass spectrometry (GC-MS) and enzyme immunoassays. These two studies showed that the measurements of F_2-isoprostanes by EIA and GC-MS are not equivalent. Therefore, comparison of levels derived using a GC-MS method which estimates concentration from a peak encompassing a number of F_2-isoprostane isomers, and levels derived from enzyme immunoassay measuring a specific isoprostane, may be inappropriate. Especially comparison of clinical results using GC-MS and EIA should be avoided.

In a related study from the same investigators (25), the effect of hypothermia on antioxidant defenses and oxidative stress was assessed in pediatric TBI. Hypothermia decreases endogenous antioxidant consumption and lipid peroxidation after experimental cerebral injury. Patients randomized to hypothermia were cooled to 32°–33°C 48 h and then rewarmed. Antioxidant status was assessed by measurements of total antioxidant reserve and glutathione. Protein thiols and F_2-isoprostane were measured in ventricular CSF on day 1–3 after injury. Multiple regression models revealed hypothermia preserved CSF antioxidant reserve. Glutathione levels were inversely associated with patient temperature. F_2-isoprostane levels peaked on day 1 after injury and progressively decreased thereafter. While F_2-isoprostane levels were approximately threefold lower in patients receiving hypothermia *vs* normothermia, this difference was not statistically significant. This was the first study demonstrating that hypothermia attenuates oxidative stress after severe TBI in infants and children (25, 99).

B. TBI and ApoE

ApoE is an important mediator of cholesterol and lipid transport in the brain and is encoded by the polymorphic gene APOE. While it may be reasonable to relate effects of ApoE to cholesterol transport, the mechanism whereby ApoE elicits these effects has not been elucidated. ApoE has been shown to reduce glial activation and CNS inflammatory response. This action is isoform-specific, with the ApoE4 isoform being less effective at downregulating inflammatory cytokines (201). A small peptide, apoE[133-149] was created from the receptor binding region that retains the ability of the native protein in downregulating inflammatory responses. Administration of apoE[133-149] was shown to significantly improve histological and functional outcome after experimental TBI (201).

While the APOE ε4 allele was first implicated as a significant risk factor for sporadic AD, a number of clinical studies have indicated that performance on neuropsychological tasks is worse in TBI patients with the APOE ε4 allele than those without it (21). While other clinical studies did not find an association of APOE ε4 allele with poorer outcome after TBI, these differences could be due to the severity of the TBI (no association in some studies with predominantly mild TBI), the neurological evaluation methods that assess the involvement of different brain regions, and the evaluation time post injury (up to 25 years after TBI) (245). In other studies, the presence of the APOE ε4 allele has been associated with poorer outcome after cardiopulmonary resuscitation and intracerebral hemorrhage (ICH), but not after ischemic stroke (324).

How the ApoE4 isoform might affect recovery after TBI is not clear, but proposed mechanisms include neurogenesis, inflammatory response, and amyloid processing or metabolism. A recent study used microarray technology to characterize the genomic response to controlled cortical impact (CCI) injury in the brains of APOE3 and APOE4 transgenic mice. Quantitatively and qualitatively significantly different profiles of gene expression in both the hippocampus and the cortex of the APOE3 mice compared to APOE4 were identified. The findings suggest that the poor recovery post-TBI in APOE4 animals and human patients is less likely due to specific activation of neurodegenerative mechanisms than a loss of reparative capability (73).

Both human postmortem and experimental studies have shown Aβ deposition and tau pathology after TBI (150). Statins have shown benefit in experimental TBI (205), but it is unknown if statin treatment affected Aβ levels. Statins overall may improve blood flow, attenuate coagulation, alter the immune system, and decrease oxidative stress, and all these factors in conjunction may show benefit in CNS pathologies (357). The development of AD-like neuropathological and biochemical changes after severe TBI suggested that TBI may be a risk factor for subsequent development of dementia. Epidemiological studies have provided discrepant findings, thus the relationship between TBI and dementia remains a topic for further investigation.

VI. Spinal Cord Injury (SCI)

Similar to TBI, SCI is the result of an initial physical trauma followed by a secondary degenerative process. The majority of SCIs result from contusive, compressive, or stretch injury rather than physical transection of the spinal cord. The initial event after SCI is depolarization and opening of voltage-dependent ion channels, and consequent massive release of neurotransmitters, including glutamate. This leads to accumulation of intracellular calcium, initiating a number of damaging events: mitochondrial dysfunction, activation of nitric oxide synthase (NOS) and PLA_2. PLA_2 releases ArAc into the COX/LOX pathways to generate eicosanoids. One consequence of mitochondrial dysfunction, activation of NOS, and COX/LOX activity is generation of free radicals (comprised of different species including RNS, ROS, and other radicals) and subsequent lipid peroxidation that is considered a major pathway of secondary injury in SCI (129).

To ascertain the role of free radicals in SCI-induced mitochondrial dysfunction, markers for oxidative stress were measured in isolated mitochondria over 24 h after SCI. A significant decline in mitochondrial function began by 12 h after injury. 3-Nitrotyrosine, HNE, and protein carbonyls showed a progressive increase, which preceded the loss of mitochondrial bioenergetics, suggesting that free radical damage may be a major mitochondrial secondary injury process (337). Pre-treatment with Edaravone, a free radical scavenger, significantly attenuated superoxide radical formation, improved motor function, and reduced lesion volume after SCI in female mice (20).

In another study of SCI in rats, administration of tempol, a scavenger of peroxynitrite-derived free radicals (nitrogen dioxide, hydroxyl radical and carbonate radical), significantly reduced peroxynitrite-mediated oxidative damage in spinal cord tissue, including protein nitration, protein oxidation, and lipid peroxidation (387). Mitochondrial dysfunction disrupts intracellular calcium homeostasis, contributing to calpain-mediated degradation of axonal α-spectrin. Tempol partially reversed SCI-induced mitochondrial dysfunction and significantly decreased α-spectrin breakdown proteins, indicating axonal protection.

Phase I enzymes predominantly catalyze formation of more polar metabolites through oxidation (*via* cytochrome enzymes responsible for mixed function oxidase activity), reduction, or hydrolysis. Phase II enzymes usually involve conjugation reactions (methylation, acetylation, sulfation, and

glucuronidation) necessary for drug metabolism or the further metabolism of phase I enzyme products. Phase II enzyme inducer is a compound which can promote the expression of antioxidative enzymes through Nrf2 activation. Generally, phase II enzyme inducers are Michael reaction acceptors, quinones and isothiocyanates, that activate transcriptional factor complexes that bind to the ARE in the promoter regions of phase II enzyme genes (153).

The loss of motor neurons due to dissection can mimic severe SCI. Increased glutamate, necrotic motor neurons, and damaged mitochondria were observed in organotypic spinal cord cultures at 48 h after dissection (198). Phase II enzyme inducers: tert-butylhydroquinone (t-BHQ), 3H-1,2-dithiole-3-thione (D3T), and 5,6-dihydrocyclopenta-1,2-dithiole-3-thione (CPDT) promoted motor neuron survival after dissection, due to increasing Nrf2 and HO-1 mRNA expression and protecting mitochondria. These results demonstrate that glutamate excitotoxicity and mitochondria damage is possibly involved in motor neuron death after SCI and phase II enzyme inducers show neuroprotective potential on motor neuron survival in SCI *in vitro* (198). The expression of antioxidant enzymes and antioxidant proteins in nervous system exhibited broad neuroprotection against injury by glutamate. Garlic has been known to have diverse biological activities, such as antithrombosis, hypolipidemia, anti-atherosclerosis, antimutagenesis, and antibacterial, and to have protective effects against ischemia–reperfusion injury. Three major components extracted from garlic are diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS). DATS caused activation of Nrf2 and Nrf2 target genes in rat spinal cord explants, and protected motor neurons against glutamate-induced excitotoxicity. These results identified DATS as a promising neuroprotective agent in neurodegeneration disease (338).

A. Clinical trials

The glucocorticoid steroids dexamethasone and methylprednisolone have been extensively used in clinical treatment of SCI. In animal studies, it was demonstrated that high dose methylprednisolone inhibited post-traumatic lipid peroxidation in spinal cord tissue. Beneficial effects secondary to inhibition of lipid peroxidation included preservation of ion homeostasis, mitochondrial energy metabolism, and attenuation of delayed glutamate release (129). It is believed that inhibition of lipid peroxidation is the principle neuroprotective mechanism of high-dose methylprednisolone and that glucocorticoid receptor-mediated anti-inflammatory effects have only a minor role (129).

Another agent that has undergone phase III clinical trials for SCI is GM1 ganglioside. Since high-dose methylprednisolone had become widely accepted for treatment of SCI, GM1 was administered only after the completion of the 24 h methylprednisolone dosing protocol. The results indicated that GM1 did not provide greater functional improvement compared to methylprednisolone alone (129). The neurosteroid, DHEA sulfate offered neuroprotection in a spinal cord ischemia model, which was believed to be mediated through $GABA_A$ receptors (181).

VII. Wallerian Degeneration

Wallerian/anterograde degeneration results when a nerve fiber is cut or crushed and the part distal to the injury (*i.e.*, the part of the axon separated from the neuron's cell nucleus) degenerates, a process in which PLA_2 plays an important role in myelin breakdown and phagocytosis (75). Wallerian degeneration occurs after axonal injury in both the peripheral nervous system (PNS) and CNS. The axonal degeneration is followed by degradation of the myelin sheath and infiltration by macrophages. The macrophages, accompanied by Schwann cells, serve to clear the debris from the degeneration. The PNS is much faster and more efficient at clearing myelin debris in comparison to CNS, and Schwann cells are the primary cause of this difference. PLA_2 expressed during the early stage of Wallerian degeneration hydrolyzes PC in myelin to LPC and ArAc. LPC can induce further myelin breakdown while eicosanoids derived from ArAc stimulate inflammatory responses. In a model of post-traumatic axonal degeneration, surgically sectioned motor nerve fibers in cats, animals were pretreated for 5 days with antioxidants α-tocopherol (vitamin E) and selenium (essential for the antioxidant enzymes glutathione peroxidase and thioredoxin reductase) prior to nerve section. Antioxidant pretreatment significantly retarded anterograde degeneration and preserved neuromuscular function. While biochemical correlates were missing, the efficacy of antioxidant treatment provided indirect evidence that ROS and lipid peroxidation may have fundamental mechanistic roles in anterograde degeneration (128, 228, 367).

VIII. Neurodegenerative Disorders with High Brain Iron

Iron has an essential function in mammalian metabolism due to the ease with which it can gain (reduction to Fe^{2+}) and lose (oxidation to Fe^{3+}) electrons. This property makes iron a useful component of hemoglobin, myoglobin, cytochromes, and various nonheme enzymes. This same property of facile electron exchange makes iron also potentially toxic by donation of electrons to oxygen, generating the highly reactive •OH through the Fenton reaction, a primary mechanism of lipid peroxidation. Consequently, mammalian cells must tightly regulate uptake, transport, and storage of iron. Iron is an essential cofactor for many proteins that are involved in normal function of neurons (Fig. 11).

Under normal physiological conditions, cells efficiently store iron in a stable form within ferritin, an effective mechanism to inhibit iron-mediated oxidations of biomolecules. Iron stored in ferritin can be released under pathological conditions by superoxide, redox cycling xenobiotics, and other small chemical reductants. Once released, iron can then initiate deleterious oxidation reactions (374).

A. Brain uptake of iron (31, 235, 330)

Iron in circulation is bound to the transport protein transferrin. The most common pathway for iron to cross the blood–brain barrier (BBB) and enter the brain is through binding of transferrin (Tf) to the Tf receptor (TfR) on brain endothelial cells followed by endocytosis. The iron-loaded Tf is internalized to the endosomes, where the acidic environment facilitates release of Fe^{3+} which is then reduced to Fe^{2+} by endosomal reductases (31). The mechanism by which iron is then transported from the interior of the endothelial cells to the interstitial fluid remains unclear. One possible mechanism is transport by the divalent metal transporter-1 (DMT-1) followed by export to the extracellular fluid by ferroportin (31). Alternatively, it has been proposed that iron is released from

transferrin on the abluminal surface of brain endothelial cells by the action of citrate and ATP (released by astrocytes) and DMT-1 is not involved in transport of iron through the endothelial cells. Instead, complexes of iron with citrate and ATP circulate in the brain extracellular fluid and may be taken up by all types of brain cells (235, 330). Iron may also be found in the interstitium bound to Tf. This Tf originates from the ventricles rather than from across the BBB. Neurons have both TfRs and DMT1. Iron-deprived neurons upregulate TfRs, which then complex with interstitial Tf-bound iron and promote endocytosis and cytoplasmic transport. Neurons might also absorb non-Tf-bound iron as they take up iron–citrate in culture. Neurons secrete excess iron not used for metabolic purposes as Fe^{2+} *via* ferroportin, although some neurons (*e.g.*, of forebrain nuclei) express ferritin and are capable of iron storage. Oligodendrocytes and astrocytes acquire solely non-Tf-bound iron. Astrocyte iron export is likely mediated by ceruloplasmin (ferroxidase activity of ceruloplasmin converts Fe^{2+} to Fe^{3+}, which binds to transferrin). Though monocytes and macrophages contain iron in order to promote free radicals as part of respiratory burst activity, brain microglia rarely contain iron. Iron can be found stored in the CNS bound not only to stable ferritin but also to more reactive substances such as neuromelanin or hemosiderin. Brain iron is likely reabsorbed back into the bloodstream by way of the subarachnoid space and CSF.

B. Neurodegeneration with brain iron accumulation (NBIA) and infantile neuroaxonal dystrophy (INAD)

Accumulation of iron in some brain regions is involved in pathology of many neurodegenerative disorders such as PD, AD, and several childhood genetic disorders, infantile neuroaxonal dystrophy (INAD) and neurodegeneration with brain iron accumulation (NBIA) (31) (Table 3). The INAD and NBIA disorders are caused by a build-up of iron in the basal ganglia, a cluster of gray matter tissue structures deep in the brain that control motor function. The iron accumulation causes the branch-like axons that transmit electrical impulses from the nerve cell body to its terminal to swell, interrupting the signal sent to other nearby nerve cells. NBIA defines a group of genetic disorders characterized by brain iron deposition and associated with neuronal death. The known causes of NBIA include pantothenate kinase-associated neurodegeneration (PKAN), neuroferritinopathy, infantile neuroaxonal dystrophy (INAD), and aceruloplasminemia. Brain MRIs from patients with molecularly confirmed PKAN, neuroferritinopathy, INAD, and aceruloplasminemia were analyzed by T2 and fast spin echo (FSE) MRI to delineate specific subtypes of neurodegeneration with brain iron accumulation (226). The term NBIA type I is now favored for the disorder formerly known as Hallervorden–Spatz syndrome (this usage has been discouraged due to criminal activities during the Third Reich) (203). NBIA type I, also known as pantothenate kinase associated neurodegeneration (PKAN) is caused by mutations in *PANK2* gene. PANK2 is the only pantothenate kinase isoform specifically expressed in the brain, which may account for the brain-specific pathophysiology of the disease (187). Pantothenate kinase catalyzes the first of five steps in synthesis of acetyl-CoA. In the second step, phosphopantothenate condenses with cysteine. When *PANK2* is mutated, phosphopantothenate is not produced and cysteine accumulates (Fig. 12). The iron chelating properties of cysteine may account for the iron overload in the brain. Cysteine undergoes rapid auto-oxidation in the presence of iron, generating ROS. Acetyl-CoA is required for phospholipid synthesis from lysophospholipid and acyl-CoA, and the defect in acetyl-CoA synthesis disrupts phospholipid homeostasis. INAD (NBIA type II) and the related Karak syndrome are caused by mu-

Table 3. Neurodegenerative Disorders Associated with Increased Brain Iron

Monogenetically caused disturbances of brain iron metabolism
Pantothenate kinase associated neurodegeneration (NBIA type 1): Mutations in PANK2 (pantothenate kinase 2)
Hereditary ferritinopathies (NBIA type 2): Mutation in exon 4 of the ferritin light chain gene on chromosome 19
Aceruloplasminemia: Autosomal recessive mutation in ceruloplasmin gene
Infantile Neuro Axonal Degeneration (INAD), (NBIA type 2): PLA2G6 ($iPLA_2$) mutations
Neurodegenerative diseases
Alzheimer's disease (AD)
Amyotrophic lateral sclerosis (ALS)
Friedreich's ataxia
Huntington's disease (HD)
Multiple sclerosis (MS)
Parkinson's disease (PD)

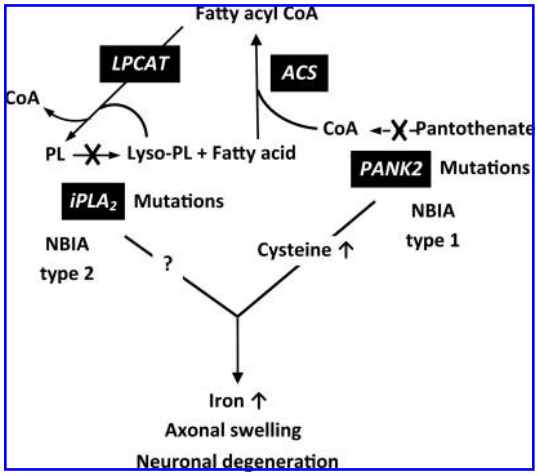


FIG. 12. Function of cytosolic calcium-independent phospholipase A_2 ($iPLA_2\beta$) and pantothenate kinase 2 (PANK2) in phospholipid metabolism (203). Levels of phospholipids are regulated by the opposing actions of hydrolysis by $iPLA_2\beta$ and synthesis. $iPLA_2$ hydrolyzes phospholipid (PL) to release a free fatty acid and lyso-phospholipid (lyso-PL). PANK2 catalyzes the first of five steps in synthesis of acetyl-coenzyme-A (CoA). In the second step, phosphopantothenate condenses with cysteine. When *PANK2* is mutated, phosphopantothenate is not produced and cysteine accumulates, indicated by ↑. Long-chain fatty acyl-CoA synthetase (ACS) conjugates CoA to free fatty acids. Lysophosphatidylcholine acyltransferase (LPCAT) synthesizes PL from lyso-PL and a fatty acid conjugated to CoA. Mutations in *PLA2G6* and *PANK2*, the genes that encode $iPLA_2\beta$ and PANK2, respectively, lead to neurodegeneration characterized by axonal swelling and iron accumulation.

tations in the gene encoding PLA2G6 ($iPLA_2$) (124, 203, 236). Neurodegeneration and dystonia are also associated with mutation in *PLA2G6* (180). NBIA covers additional disorders of high brain iron, including neuroferritinopathy and aceruloplasminemia. The mutations underlying both NBIA types I and II cause defects in lipid metabolism, however, how these defects relate to iron accumulation is not known. It has been hypothesized that lipids are required for proper iron metabolism or that specific lipid species may regulate iron transport proteins. Also, the relationship of brain iron accumulation to neurodegeneration and progression of NBIA is unclear: whether iron accumulation is a causative factor in neurodegeneration or a result. It has been suggested that altered lipid metabolism is the primary cause of NBID, and that iron accumulation is a secondary and variable phenomenon not directly related to the cellular dysfunction (301).

The pathological changes found in PKAN occur primarily in tissues with normally high energy demands and primary sites of injury, especially the globus pallidus and retina, specifically the photoreceptors. In the globus pallidus, iron is normally highly concentrated and redox active (138). Photoreceptors are intensively membranogenic, and lipid peroxidation damage from free radicals and light exacerbates cellular oxidative stress (138). *PLA2G6* encodes group VI $iPLA_2$ that is important in phospholipid remodeling, ArAc release, leukotriene and prostaglandin synthesis, generation of lipid peroxides, and apoptosis (319). Membrane repair and remodeling represents a compelling common pathway in which the protein products of both *PANK2* and *PLA2G6* play critical roles.

C. Ferritinopathy

Mammalian ferritins are heteropolymers composed of 24 polypeptide subunits that contain variable proportions of the light (FTL) and heavy (FTH1) polypeptide subunits. Hereditary ferritinopathy (also referred to as neuroferritinopathy) is an autosomal dominant extrapyramidal disease caused by mutations in exon 4 of the ferritin light chain gene on chromosome 19. Ferritinopathy is characterized by an increase of iron and ferritin in the extracellular spaces and cytoplasm of cells in the basal ganglia of affected individuals and is associated with low serum ferritin levels (365). Symptoms usually start between the third and sixth decade of life and include rigidity, choreoathetosis, dystonia, and spasticity. In a report on a family diagnosed with hereditary ferritinopathy (209), a mutation was found involving a C insertion at nt646-647 in exon 4 in the ferritin light chain gene, resulting in a longer than normal protein. Postmortem analyses showed that both neurons and glia of the posterior putamen and cerebellum displayed highly distinctive, swollen to vacuolated nuclei containing ferritin and iron. There was a nearly 40-fold increase in iron in the putamen, which appeared to be in both the Fe^{3+} and Fe^{2+} states. Neurons of the putamen demonstrated immunoreactivity for both lipid peroxidation products MDA and HNE. A follow-up study of the patients (269) showed that the heme-containing proteins neuroglobin and cytoglobin were overexpressed in the putaminal neurons and glia, demonstrating another source for abnormal iron, heme, and protein inclusions in hereditary ferritinopathy. The swollen to vacuolated nuclei in the putamen were positive for activated caspase-3 as well as p53. These studies support a role for oxidative stress and lipid peroxidation due to excessive iron accumulation in the pathogenesis of this disease.

D. Aceruloplasminemia

Aceruloplasminemia is an autosomal recessive disorder caused by mutations in the gene encoding ceruloplasmin, which converts Fe^{2+} to Fe^{3+} in astrocytes through ferroxidase activity. The typical clinical presentation is a triad of neurological disease, retinal degeneration, and diabetes mellitus. The neurological involvement is characterized by neurodegeneration and excessive iron accumulation primarily in the basal ganglia and retina, and to a lesser extent, in the cerebellum and cortex. Due to its ferroxidase activity, ceruloplasmin has an essential role in iron metabolism in the brain in oxidizing Fe^{2+} to Fe^{3+}. Since only the oxidized Fe^{3+} form binds to transmembrane carriers and iron-storage proteins such as ferritin, loss of ceruloplasmin function results in abnormally high intracellular iron concentrations. The increase in nonprotein bound iron such as Fe^{2+} leads to generation of excessive ROS and increased lipid peroxidation (32, 396).

IX. Alzheimer's Disease (AD)

AD is a progressive brain disorder affecting regions that control memory and cognitive functions, gradually destroying a person's memory and ability to learn, reason, communicate, and carry out daily activities. One of the hallmarks of AD is overproduction of amyloid β-peptide ($A\beta$), resulting in the formation of plaques. A two-step cleavage of the neuronal membrane protein amyloid precursor protein (APP) (88) results in two products, $A\beta40$ and $A\beta42$. Strong evidence for the role of $A\beta$ in the pathogenesis of AD was provided by the observation that mutations in APP or the enzymes that cleave it lead to overproduction of $A\beta42$ and rapid progression of the disease (210). However there is also evidence against the amyloid hypothesis in AD due to the failure of $A\beta$ immunotherapy (114, 210). The second hallmark of AD is formation of neurofibrillary tangles due to hyperphosphorylation of tau protein. It is the tangles and not plaques that are directly related to mitochondrial dysfunction in ROS generation (135, 136).

Transgenic mouse models of AD require two or more mutations to reproduce all the physical features of AD ($A\beta$ plaques and tau tangles), however these models have not provided any leads to the relationship between $A\beta$ plaques and tau tangles. Most mouse models of AD are considered limited or incomplete as they do not exhibit the extent of pathology seen in AD patients (210): several models develop amyloid deposition but fail to develop neurofibrillary tangles that are an essential hallmark of AD (306). Neuritic atrophy is found in some transgenics, but of nearly one dozen mouse models, only one has reported loss of neurons that is characteristic of AD (140). Some tau models develop severe memory deficits associated with AD but express little amyloid protein (210). Other questions remain: Are the familial and sporadic forms of AD distinct? Mouse models are based on familial AD (the rare form of the disease) and may not model the common sporadic form. The triple transgenic mouse (APP/PS1/tau) studies raise the possibility that a multitargeted approach (*i.e.*, simultaneously targeting $A\beta$ and tau) may provide the most significant clinical benefit for the treatment of AD (252).

There is growing evidence that cholesterol is of particular importance in development and progression of AD. Identification of the gene encoding the variant ApoE4 (APOE ε4 allele) as a significant risk factor for sporadic AD provided evidence for a role of cholesterol in the pathogenesis of AD (272). Elevated cholesterol levels increase Aβ in cellular and animal models, and drugs such as statins and BM15.766 that inhibit cholesterol synthesis lower Aβ levels (134, 272, 377). Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase that initiates cholesterol and isoprenoid lipid synthesis while BM15.766 inhibits cholesterol synthesis at the ultimate step (134). Prospective trials evaluating statin therapy, however, did not demonstrate improvement in cognitive function in AD patients (53).

Cholesterol is needed to make the cellular membrane micro-domains referred to as lipid rafts. APP, β-secretase, γ-secretase complex, and neutral sphingomyelinase (NSMase) are present in the lipid rafts that are rich in cholesterol and SM. Genetic mutations in APP or presenilins (part of the γ-secretase complex) increase production of Aβ42. Recent studies suggest that Aβ40 inhibits HMG-CoA reductase while Aβ42 activates NSMase and increases ceramide production, which can accelerate the neurodegenerative process (127, 221). It is unclear what regulates the cleavage of APP to Aβ42 *vs* Aβ40.

Cholesterol is the precursor for biosynthesis of neurosteroids. In AD brains, a general trend was observed towards decreased levels of all steroids, with significantly lower amounts of pregnenolone and dehydroepiandrosterone. These lower levels correlated with increased amounts of β-amyloid peptides and phosphorylated tau proteins. It is not known whether these neurosteroid deficiencies contribute to or result from AD pathology, but since many neurosteroids have neuroprotective actions, their lower levels may contribute to Aβ neurotoxicity (369). Studies have indicated that allopregnanolone is the most effective neurosteroid for neuroprotection. Allopregnanolone also stimulated neurogenesis by increasing expression of genes that promote mitosis and inhibiting expression of those that repress cell proliferation, and may be a promising therapy for promoting cellular regeneration in AD and other neurodegenerative disorders (369).

A. AD and iron

Substantial iron deposits have been found in AD brains at sites most severely affected by neurodegeneration, indicating that iron could be a major cause of oxidative stress in AD (298, 326). Senile plaques and neurofibrillary tangles accumulate iron and are major sites of catalytic redox activity. Aβ binds iron, facilitating its aggregation and increasing its toxicity (can initiate •OH generation causing oxidative stress), which can be alleviated by iron chelators (225). Iron may also impact on plaque formation by affecting APP processing and the ability of α-secretase to cleave APP. 5-Chloro-7-iodo-8-hydroxyquinoline (clioquinol; a conventional metal (Cu, Zn) chelator and an ionophore), the discarded indigestion drug (54) that might move trace metals outside the cell where their accumulation may cause neuronal damage, provided some benefit in AD models. Oral treatment with clioquinol in Tg2576 mice (AD transgenic model) resulted in a significant 49% reduction in cortical amyloid deposits and improved health compared to untreated controls (52). Clioquinol can also prevent both ubiquitination and hydroxylation of hypoxia inducible factor-1α (HIF-1α) and may offer protection independent of iron chelation (65).

B. AD, oxidative stress, and lipid peroxidation

mRNA expression of pro-inflammatory $sPLA_2$ IIA was upregulated in AD brains compared to nondementia elderly brains. $sPLA_2$ IIA immunoreactive astrocytes in AD hippocampus were associated with Aβ plaques (238). $sPLA_2$ could contribute to lipid peroxidation through the release of ArAc. Studies demonstrating increased lipid peroxidation in AD support a role for oxidative damage in this disorder (380). Recent studies showed increased cytosolic PLA_2 ($cPLA_2$, group IVA) activity in AD patients and mice expressing human amyloid precursor protein (hAPP). Aβ caused dose-dependent increase in $cPLA_2$ phosphorylation in neuronal cultures. $cPLA_2$ inhibition decreased Aβ-induced toxicity, Aβ-dependent learning and memory deficits, behavioral alterations, and premature death (294). HNE and acrolein levels were increased in the brain tissue from patients with mild cognitive disorder and early AD, indicating that lipid peroxidation occurs early in the pathogenesis of AD (380). Acrolein, by far the strongest electrophile among all α,β-unsaturated aldehydes, reacts with DNA bases to form cyclic adducts, the major exocyclic adduct being acrolein-deoxyguanosine, which was elevated in brain tissue from AD patients (197). ROS may also play a role in amyloid deposition in AD as oxidizing conditions cause protein cross-linking and aggregation of Aβ peptides, and also contribute to tau protein aggregation (213). Aβ aggregation stimulates ROS production, which may lead to cyclic or self-propagating oxidative damage. The DHA metabolite neuroprotectin D1 promoted neuronal survival and anti-apoptotic pathways that attenuated Aβ42 neurotoxicity (200).

Expression profiles of antioxidant enzymes and Nrf2 have been examined in AD brains. Catalase activity was decreased whereas HO-1 and NQO1 were increased. Large pyramidal neurons, which are susceptible to degenerative processes in AD, showed increased expression of cytosolic Cu/Zn SOD (SOD1) (76). In AD brain, Nrf2 is predominantly localized in the cytosol of hippocampal neurons. Nuclear Nrf2 expression levels, determined by immunoblotting, are significantly decreased in AD cases, indicating that Nrf2-mediated transcription is not induced in neurons in AD despite the presence of oxidative stress (278). Nonetheless, expression of Nrf2-ARE regulated proteins, such as Gpx and HO-1, is increased in AD brains. These discrepancies could be due to examining expression levels at different stages of AD pathology, expression levels *vs* activity, or cell-specific expression. The accumulation of dysfunctional DJ-1 may play an important role in decreasing Nrf2 levels in AD (76).

X. Parkinson's Disease (PD)

PD is the second most prevalent age-related neurodegenerative disease after AD and over 1 million people in the United States are affected (229). PD is characterized by selective degeneration of dopaminergic neurons of the substantia nigra, which are necessary for motor function, and formation of protein aggregates called Lewy bodies in surviving neurons (199). Loss of these dopaminergic neurons causes the PD symptoms of bradykinesis (slowing and di-

minished range of movements), akinesia (difficulty in initiating voluntary movement), abnormal postural reflexes, a rhythmic 5–7 Hz tremor in patients at rest (the characteristic "pill rolling movement"), and rigidity (378). About 95% of PD cases are late-onset sporadic, whereas the remaining 5% are familial and characterized by early onset. Familial PD has been linked with mutations in genes *PARK1*, *PARK2*, and *PARK5* that encode α-synuclein, parkin (an E3 ubiquitin ligase involved in degradation of misfolded or damaged proteins by the ubiquitin–proteasome pathway) and ubiquitin carboxy-terminal hydrolase L1 (believed to recycle ubiquitin by cleaving ubiquitinated proteins), respectively (199, 378). Epidemiological studies have offered few clues for causative factors in PD, and despite many years of research, the biochemical mechanisms and genetic/epigenetic factors underlying the initiation and progression of PD remain elusive. Currently, oxidative stress remains the leading theory for development of PD (229). Dopamine can auto-oxidize at normal pH or be metabolized by monoamine oxidase, giving rise to superoxide radicals and hydrogen peroxide. Because of its potential toxicity, dopamine is normally sequestered into synaptic vesicles which have a low pH and lack monoamine oxidase, providing a stable environment. It has been proposed that defective sequestration of dopamine into vesicles is a key event leading to the demise of dopaminergic neurons in PD (199). The accelerated metabolism of dopamine by monoamine oxidase B in dopaminergic neurons can result in excessive production of superoxide anion radicals and hydrogen peroxide. Other sources of ROS in PD include activation of phospholipases including $cPLA_2$ and induction of NADPH oxidase in activated microglia (229). Microglia are the resident immune cells in the brain, and when activated produce large amounts of ROS through the NADPH oxidase system in their role as phagocytic cells. PD patients can have more than six times the number of reactive microglia compared to controls. In support of the role of oxidative stress in PD, marked increases in 8-hydroxy-2′-deoxyguanosine (8-OHdG), a hydroxyl radical-damaged guanine nucleotide, were found in PD brain regions (213). Levels of MDA and lipid hydroperoxides were found to be up to 10-fold higher than normal in the substantia nigra of PD patients (80, 81). An average of 58% of nigral neurons stained positive for HNE in PD brains, in contrast to 9% in control subjects (395). Levels of glutathione, a major cellular antioxidant defense, are markedly decreased in the substantia nigra of PD patients.

Studies have demonstrated strong upregulation of Nrf2-ARE-regulated proteins such as NQO1 and HO-1 in post-mortem brain samples. This increased expression appears to be restricted to astro(glial) cells and is virtually absent in dopaminergic neurons. In contrast, Nrf2 localization studies in the substantia nigra of PD brain demonstrated strong nuclear immunoreactivity in neurons, indicative of Nrf2 activation (76). Together, these data suggest that factors may be operating to prevent Nrf2-ARE gene transcription in dopaminergic neurons. This lack of ability to upregulate an antioxidant response could render dopaminergic neurons particularly susceptible to oxidative damage.

A. PD and iron

The level of iron is significantly higher in the normal substantia nigra compared to other brain regions owing to its binding affinity to neuromelanin (199). Iron levels have been demonstrated to be increased by ~35% in the Parkinsonian substantia nigra, with iron levels increasing with the severity of neurological changes. This is associated with a profound inflammatory response characterized by proliferation of reactive microglia and an increase in pro-inflammatory cytokines, interleukins 1 and 6, and tumor necrosis factor α. There is a shift in the Fe^{3+}/Fe^{2+} ratio from 2:1 to almost 1:2, with larger amounts of Fe^{2+}, suggesting that changes in iron content may contribute to deleterious oxidative stress.

In mammals, two homologous cytosolic regulatory proteins, iron regulatory protein 1 (IRP1/Aco1) and iron regulatory protein 2 (IRP2/Ireb2), sense cytosolic iron levels and post-transcriptionally regulate iron metabolism genes, including TfR1 and ferritin H and L subunits. Mice with a targeted disruption of the gene for IRP2 developed tremors and weakness in the limbs (184). Significant accumulations of iron in white matter tracts and nuclei throughout the brain precede onset of neurodegeneration and movement disorders, suggesting that misregulation of iron metabolism may contribute to the pathogenesis of human neurodegenerative diseases. Humans with a mutation in this IRP2 gene might have been incorrectly diagnosed as having PD or some other neurological disorder that causes tremors and impairs movement. Treatment of $IRP2^{-/-}$ mice with a stable nitroxide, Tempol, markedly attenuated progression of neuromuscular impairment (115). The researchers are also studying whether treatment with Tempol can reverse nervous system damage that has already occurred (115). Tempol works by activating iron IRP1. IRP1 and -2 have the same function—both proteins regulate how much iron is in a cell.

B. Dopaminergic neurotoxins

Epidemiological studies have identified age as the greatest risk factor for development of PD, followed by family history, head injuries, and exposure to herbicides (308). However, the majority of PD patients do not report a family history of PD (229). Environmental factors have been shown to contribute to the development of PD as geographic areas where herbicides containing paraquat overlap with areas where PD is prevalent, and people exposed to paraquat develop PD-like symptoms.

Several other neurotoxins have been shown to induce PD-like symptoms. The first dopaminergic neurotoxin discovered was 6-hydroxydopamine (6-OHDA), which has been used for over 30 years in experimental models of PD (46). 6-OHDA is also taken up by catecholaminergic neurons and can damage these neurons throughout the body (229). Upon transport to neurons, 6-OHDA generates free radicals through oxidation similar to dopamine, and inhibition of mitochondrial complex I. Accumulation of endogenous 6-OHDA has been shown in PD patients (302).

The neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was discovered as a contaminant in synthetic heroin when several drug abusers presented with severe PD-like symptoms (229, 302). These findings provided the first evidence that environmental factors such as exogenous toxins could be involved in the pathophysiology of PD (229). The MPTP metabolite MPP^+ binds with high affinity to dopaminergic transporters, then taken up by nigrostriatal neurons where it inhibits mitochondrial oxidative phosphorylation,

causing neuronal death. MPP^+ can be taken up into dopaminergic vesicles *via* its high affinity for vesicular monoamine transporter 2. In addition to depleting intracellular ATP stores, MPP^+ can cause redistribution of dopamine to the cytoplasm, promoting dopamine-dependent oxidative stress (199). MPTP neurotoxicity is also commonly used as an animal model for PD. Another factor responsible for generation of free radicals and lipid peroxidation in PD is believed to be phospholipases activation in substantia nigra, supported by the fact that $cPLA_2$-deficient mice are resistant to MPTP-induced neurotoxicity (94). Although MPTP produces virtually all the symptoms of PD, strictly it is not PD.

The two most common toxin-induced PD models, 6-OHDA and MPTP, also have two points of weakness (a) both neurotoxins selectively and rapidly destroy catecholaminergic neurons, whereas in humans the PD pathogenesis follows a progressive course over decades (302), and (b) Lewy bodies characteristic of PD are not formed in either of these models (229). MPTP administration resulted in loss of dopamine transporter levels in the striatum of Nrf2 null mice compared to wild type. Nrf2 activator 3H-1,2-dithiole-3-thione (D3T) provided partial protection against MPTP toxicity. The protective effect was not due to change in MPTP metabolite. D3T administration to Nrf2 null mice did not protect against MPTP toxicity, indicating that Nrf2 pathway is necessary for D3T-mediated reduction in MPTP toxicity (51). In a recent study (62) transgenic mice with Nrf2 under the regulation of astrocytic-specific promoter for GFAP (GFAP-Nrf2) on a both wild type as well as Nrf2 null background were administered MPTP. In the Nrf2 null background only astrocytes expressed Nrf2. MPTP-mediated toxicity was abolished in GFAP-Nrf2 mice irrespective of the background indicating that Nrf2 expression restricted to astrocytes is sufficient to protect MPTP toxicity. Astrocytes expressing Nrf2 also provided significant protection against 6-OHDA (147).

C. PD and α-synuclein

PD is associated with the presence of Lewy bodies containing insoluble aggregates of α-synuclein, a pre-synaptic protein of unknown function, in association with other proteins, including ubiquitin, proteasome subunits, heat-shock proteins, and neurofilaments. Lewy bodies are also found in the normal ageing brain and in the brains of patients with other neurodegenerative diseases, such as AD and dementia with Lewy bodies (199). Full-length α-synuclein normally occurs in a natively unfolded state, but at high concentrations can form oligomers of β-pleated sheets, called protofibrils. Whether Lewy bodies are neuroprotective or cytotoxic is still a matter of debate. Since Lewy bodies are found in surviving neurons, it has been suggested that sequestration of protofibrils into such 'aggresomes' is a means by which the cell protects itself from toxicity, and cells containing Lewy bodies represent those that have managed to evade the toxic mechanisms involved in PD (199). However, other studies in patients with dementia with Lewy bodies report a correlation between numbers of cortical Lewy bodies and the degree of clinical symptoms. Although sequestering potentially toxic proteins might initially be beneficial to dopaminergic neurons, such inclusions could become toxic once they occupy a large part of the cell body (199). The relationship between oxidative stress and formation of Lewy bodies in PD is still an open question (199). Genetics of Parkinson's and Lewy body disease as well as disparate syndromes such as Hallevorden–Spatz and Niemann–Pick C diseases and possible ceramide connection has been reviewed recently (47, 133).

α-Synuclein transfection of cultured human fetal dopaminergic neurons resulted in accumulation of soluble protein complexes containing α-synuclein, increased ROS generation, and apoptosis. α-Synuclein transfection of nondopaminergic human cortical neurons did not cause cell death (388). Expression levels of α-synuclein were similar between transfected dopaminergic and nondopaminergic neurons, indicating dopamine-specific neurotoxicity of α-synuclein. The free radical spin trap phenyl-*N*-butyl-nitrone and the antioxidant vitamin E both inhibited ROS generation and apoptosis in transfected dopaminergic neurons, indicating ROS-mediated induction of apoptosis by α-synuclein. Inhibition of tyrosine hydroxylase with α-methyl-*p*-tyrosine attenuated dopamine levels and abolished α-synuclein-induced apoptosis (388). α-Synuclein inclusions were not detected in transfected dopaminergic neurons, indicating that accumulation of soluble α-synuclein is sufficient to induce apoptosis. These studies suggested that accumulation of soluble α-synuclein protein complexes could render endogenous dopamine toxic, a possible mechanism for the selective neuronal loss in PD.

Recently, the presence of PUFA was linked to the appearance of soluble oligomers of α-synuclein that ultimately promote the formation of insoluble α-synuclein aggregates (373). DHA was shown to stimulate oligomerization of α-synuclein, and DHA levels were elevated in PD brains (314), suggesting that DHA could have a role in formation of α-synuclein aggregates. On the other hand, DHA reduced levodopa-induced dyskinesias in MPTP-treated monkeys (293). This discrepancy for the role of DHA in PD warrants further investigation.

D. Treatment of PD

By the time symptoms of PD appear, 50%–80% of dopaminergic neurons have already been lost (302). Thus, identifying the underlying causes of PD and developing strategies aimed at preventing its onset would seem to be of high priority. The dopamine prodrug levodopa remains the treatment option for PD, however, long-term levodopa therapy leads to dyskinesia. Alternatives for early PD therapy include monoamine oxidase B inhibitors, dopamine agonists, catechol-*O*-methyltransferase (COMT) inhibitors, and amantadine (137). The mechanism of action of amantadine remains unknown; however, it has been suggested to have anticholinergic properties in addition to acting as an NMDA receptor antagonist to increase dopamine release and inhibit its reuptake. COMT inhibitors are used in combination with levodopa, and act peripherally to increase the pool of available levodopa, optimize its transport to CNS, and decrease the side effects of levodopa by allowing lower doses (41). CDP-choline increases tyrosine hydroxylase activity and has been used in combination with levodopa for PD treatment. CDP-choline showed functional improvements and allowed levodopa to be administered at lower doses, decreasing its side effects (2). The neurosteroid pregnenolone enhances neuronal dopamine release and may provide a therapeutic option for PD. Recent studies showed that coenzyme Q_{10} (CoQ_{10}, an essential cofactor of the electron transport chain where it accepts electrons from complex I and complex II) and creatine (an important

compound in buffering energy imbalances) are promising agents for neuroprotection in neurodegenerative diseases *via* their effects on improving mitochondrial function and cellular bioenergetics and their properties as antioxidants. These two compounds in combination provided additive neuroprotective effects in PD (MPTP toxicity) and HD models (3-nitropropionic acid in rat and R6/2 transgenic mouse) (393). The two agents produced additive effects against loss of tyrosine hydroxylase activity (in substantia nigra) and dopamine (striatum). This combination also reduced lipid peroxidation and α-synuclein accumulation in substantia nigra neurons of MPTP treated mice.

XI. Niemann–Pick Diseases (NPD)

NPD are genetic pediatric neurodegenerative conditions characterized by specific disorders in lipid metabolism and are categorized as types A, B, and C. NP Types A and B (NPA and NPB) are caused by deficiencies in acidic sphingomyelinase (ASMase) (303). NPA, the most common type, is caused by a nearly complete lack of ASMase. Over 100 mutations in *SMPD1*, the gene encoding ASMase, have been described in ASMase-deficient NPD. Patients with NPA display enlarged livers and spleens, failure to thrive, and abnormal neurological examinations within the first 6 months of life. Neurodegeneration proceeds rapidly and leads to death within 3 years. Since ASMase is localized to the lysosomes, NPA results in accumulation of SM and is classified as a lysosomal storage disorder (104). People with NPB have ~10% of the normal level of ASMase, generally have little or no neurologic involvement, and frequently survive into adulthood. Enlarged livers and spleens are the most consistent clinical findings in NPB. The most consistent laboratory finding is a highly atherogenic lipid profile (*i.e.*, high triglycerides and LDL-cholesterol; low HDL-cholesterol), and a history of coronary artery disease may be found. The phenotypic spectrum among these individuals varies widely, and the age of onset may be from early childhood to adulthood. Intermediate cases have been reported, and thus NPB may be best thought of as a single entity with a spectrum of phenotypes (303). Tricyclodecan-9-yl potassium xanthate (D609), a widely known PC-phospholipase C inhibitor, also inhibits SM synthase (183) and may help prevent accumulation of SM in NPB, a possibility not yet tested.

A recent review updates the disorders of NPC from clinical features to animal models and molecular mechanisms (342). NP type C (NPC) is always fatal but it differs from NPA and NPB at the biochemical and genetic level. NPC is caused by mutations in either the *NPC1* or *NPC2* genes, with about 95% of human NPC cases due to mutations in *NPC1* (360). NPC1 and NPC2 proteins are involved in transport of lipids, particularly cholesterol, from the late endosomes/lysosomes (223, 335). It appears that NPC2 protein could mobilize cholesterol rapidly in an acidic environment enriched in endosome/lysosome (E/L)-specific phospholipids (335). Deficiencies in these proteins result in lysosomal accumulation of cholesterol and other lipids. In NPC, cholesterol accumulates in all tissues except the brain, where total cholesterol levels decrease with age (360, 386). Since 70–80% of cholesterol in the brain is contained in myelin, the extensive demyelination that occurs in NPC probably accounts for the net loss of cholesterol in the brain, which would likely mask accumulation of cholesterol in neurons or astrocytes. In a mouse model of NPC, significant neuronal accumulation of cholesterol was shown by postnatal day 9 when only mild signs of neurodegeneration were detectable (284).

Ras-related protein in brain (Rab GTPase) were identified as essential regulators of membrane trafficking (289, 307). Overexpression of these vesicular transport proteins Rab7 and Rab9 decreased the lipid traffic by increasing the delivery of sphingolipids to Golgi and unesterified cholesterol to endoplasmic reticulum (36, 66). It was unclear whether these Rab proteins can transport cholesterol to mitochondria.

Currently there are no treatments for NPC. Despite the accumulation of cholesterol in late endosomes/lysosomes (E/L), neither cholesterol lowering agents (107, 344) nor dietary measures slowed the progression of the disease. These studies suggest that cholesterol accumulation *per se* is not the major contributor to the pathogenesis of NPC, but that disrupted cholesterol transport within the cell to the endoplasmic reticulum and mitochondria for cholesterol esterification and synthesis of neurosteroids may be the critical factor. Neurosteroids and enzymes involved in steroid synthesis were significantly reduced in NPC1-deficient mice. Administration of the neurosteroid allopregnanolone to NPC1-deficient mice increased the lifespan, delayed the onset of neurological impairment, increased survival of Purkinje and granular cell survival in the cerebellum, and reduced cortical ganglioside accumulation (125), suggesting that neurosteroid therapy might be a treatment option for NPC (50, 125).

Recent studies have provided evidence for a role of ROS in NPC. Concentrations of ROS and lipid peroxidation were higher in fibroblasts from NPC patients than in fibroblasts from normal subjects. Fibroblasts from NPC patients were more susceptible to cell death through apoptosis after an acute oxidative insult. This process is mediated by activation of the NF-κB signaling pathway. Knockdown of NPC1 mRNA both in normal fibroblasts and in human SH-SY5Y neuroblastoma cells caused increased ROS concentrations. Allopregnanolone treatment of fibroblasts from NPC patients or of NPC1 knockdown cells reduced the levels of ROS and lipid peroxidation, and prevented peroxide-induced apoptosis and NF-κB activation. These findings suggested that oxidative stress might contribute to the NPC disease and allopregnanolone might be beneficial in the treatment of NPC by restoring the intracellular redox state (403).

In addition to accumulating cholesterol, NPC1-deficient cells also accumulate gangliosides and other glycosphingolipids, and neuropathological abnormalities in NPC disease closely resemble those seen in primary gangliosidoses. Treatment of NPC mice with N-butyldeoxynojirimycin, an inhibitor of glycosphingolipid synthesis, increased the average life span, and reduced ganglioside accumulation and neuropathological changes (360).

The exclusive endosomal phospholipid LBPA may become limiting upon pathological cholesterol accumulation in NPC disease conditions. Excess cholesterol accumulation was reverted by the exogenous addition of LBPA to NPC cells (63a).

XII. Peroxisomal Biogenesis Disorders

Peroxisomes are small metabolic organelles that contain >50 enzymes that participate in β-oxidation of 2-methyl branched-chain and very-long-chain fatty acids, synthesis of

ether lipids, and oxidation of D-amino acids and polyamines (22). Mammalian cells contain large numbers of these organelles, ranging in number from a few hundred to a few thousand per cell. Peroxisomal enzymes are synthesized in the cytoplasm and imported post-translationally across the peroxisomal membrane; almost all peroxisomal enzymes contain a type-1 peroxisomal targeting signal at their extreme carboxyl terminus (121). Deficiencies in specific peroxisomal enzymes are the cause of a number of human diseases, most of which involve neurological impairment. The most severe peroxisomal disorders are caused by defects in peroxisomal biogenesis, resulting in simultaneous loss of several peroxisomal metabolic functions. Three general phenotypes have been recognized: (a) lack of peroxisomal membrane- and matrix-protein import, resulting in the absence of peroxisomes in the cell, (b) defects in peroxisomal matrix protein import with no defect in membrane synthesis or import of membrane proteins, and (c) defects in peroxisomal abundance but no defect in import of either matrix or membrane proteins (121). Genes required for peroxisome biogenesis are referred to with the acronym PEX, and their products are called peroxins. At present, 15 *PEX* genes have been identified in humans (121).

A. X-linked adrenoleukodystrophy (ALD)

X-ALD is a demyelinating peroxisomal disorder caused by mutations in the gene ABCD1 that maps to Xq28 and codes for a peroxisomal membrane protein that is a member of ATP-binding-cassette transporter super family, resulting in impairment of β-oxidation of very-long-chain fatty acids (VLCFA) leading to a fatal demyelinating disorder in boys (85, 372). One in 20,000 males is affected by ALD. Impaired VLCFA metabolism (β-oxidation) leads to accumulation of VLCFA, particularly tetracosanoic acid (24:0) and hexacosanoic acid (26:0) (Table 4), and may trigger the onset of this disease (355). In the brain, the cholesterol ester fraction contains an excess of VLCFA in regions where there is inflammatory demyelination, while the cholesterol ester composition was normal in histologically intact brain regions (237). It was concluded from these findings that VLCFA excess in brain cholesterol esters is the consequence rather than the cause of demyelination. It has been proposed that the primary impairment involves an as yet undefined mitochondrial defect; however a detailed study of mitochondrial metabolism in ALD muscle tissue failed to demonstrate such a defect. Another hypothesis is that excess VLCFA cause membrane abnormalities that contribute to the pathogenesis of ALD. Biophysical studies have shown that inclusion of 26:0 in a model membrane disrupts the membrane structure (237).

Lovastatin (HMGCoA reductase inhibitor, cholesterol lowering drug) normalized VLCFA levels in two out of three clinical studies (35). It was shown that overexpression of *ABCD2*, the closest relative of *ABCD1*, significantly reduced the accumulation of VLCFA in *ABCD1* cells. The *ABCD2* gene was induced in cultured human fibroblasts and monocytes upon sterol depletion *via* activation of sterol regulatory element-binding proteins (SREBPs), a family of transcription factors that regulate cholesterol and fatty acid metabolism (372). Thus lovastatin may reduce VLCFA accumulation by sterol depletion and upregulation of *ABCD2* expression. Lorenzo's oil, a mixture of erucic (C22:1) and oleic acids, is used therapeutically to normalize plasma VLCFA levels. Mustard seed oil, used in cooking, has naturally high levels of erucic acid and can lead to an elevation similar to that observed during Lorenzo oil therapy (http://www.x-ald.nl/diagnosis.htm). Recent studies reported the increased NO synthesis, NADPH oxidase activity, and inflammatory mediators in X-ALD lymphoblast cell line under nonstimulated conditions (116, 261, 354). These lymphoblasts contain seven times higher levels of C26:0 fatty acids compared to control. Lovastatin as well as sodium phenylacetate and the combination of both reduced these fatty acids levels (354). NO synthesis was increased in this cell line as well as the NADPH oxidase subunit gp^{91} protein levels, but the mRNA levels were unaltered. TNF-α and IL-1β levels were also elevated. Lovastatin or sodium phenylacetate markedly decreased the NO synthesis.

Table 4. Plasma Very-Long-Chain Fatty Acid Levels in X-ALD Patients (355)

$\mu mol/L$	*Normal*	*Female carriers*	*Males with X-ALD*
C24.0	61 ± 14	98 ± 16	95 ± 30
C26.0	0.67 ± 0.13	1.54 ± 0.72	2.94 ± 0.87
C26.1	0.57 ± 0.16	1.19 ± 0.54	2.06 ± 0.84

X-ALD, X-linked adrenoleukodystrophy.

B. Zellweger syndrome

Zellweger syndrome is the most severe of the peroxisomal biogenesis disorders (PBD), and is characterized by craniofacial and eye abnormalities, neuronal migration defects, hepatomegaly, and chondrodysplasia punctata (332). Zellweger infants rarely survive past 6 months.

Two mouse models of Zellweger syndrome have been developed by targeted disruption of *PEX2* or *PEX5* peroxisomal assembly genes encoding targeting signal receptor peroxins for recognition and transport of a set of peroxisomal enzymes, including those of peroxisomal β-oxidation (145).

XIII. Friedreich's Ataxia

Friedreich's ataxia, affecting about 1 in every 50,000 people in the United States, is an autosomal recessive inherited disease that causes progressive damage to the nervous system resulting in gait disturbance, speech problems, and heart disease. The term "ataxia" refers to coordination problems such as clumsy or awkward movements and unsteadiness. Friedreich's ataxia results from the degeneration of nerve tissue in the spinal cord and of nerves that control muscle movement in the arms and legs. The spinal cord becomes thinner and nerve cells lose some of their myelin sheath. MRI of the CNS usually shows cervical cord atrophy associated with atrophy of the cerebellum and brain stem. A homozygous unstable expansion of a GAA-triplet repeat sequence in the first intron of the gene encoding the mitochondrial protein frataxin has been detected in 97% of Friedreich's ataxia patients; the remaining 3% are heterozygous for an expansion in one allele and a point mutation in the other. Several studies provide evidence that frataxin is necessary for iron incorporation in iron–sulfur clusters and heme biosynthesis, sustaining mitochondrial energy production, and may also detoxify surplus iron (32). Cells lacking frataxin accumulate

iron in the mitochondria (Fig. 11) The combination of iron accumulation and abnormal respiratory chain function and evidence for lipid peroxidation has lead to the hypothesis that Friedreich's ataxia is caused, at least in part, by oxidative stress. However, this theory has been questioned by the observation that complete frataxin deficiency does not induce oxidative stress in neuronal tissue (32).

XIV. Multiple Sclerosis (MS)

MS is an inflammatory demyelinating autoimmune disease affecting the CNS, but its underlying cause remains elusive. The mechanism that triggers the immune system to attack the myelin as foreign remains elusive, and it has been questioned whether inflammatory demyelination is primary or secondary to the disease process (347). In MS, the immune system attacks the myelin sheath of nerve cell fibers in the brain and spinal cord. MS displays four variable levels of disability: (a) Relapsing/remitting MS involves temporary periods of disability followed by full or partial recovery due to remyelination of axons. The majority of MS patients are initially diagnosed with relapsing/remitting MS. (b) Progressive relapsing MS patients show significant recovery immediately following a relapse but with a gradual worsening of symptoms. Progressive relapsing MS affects ~5% of patients, and may be a variant of primary progressive MS. (c) Primary progressive MS is a gradual progression of the disease from its onset with no superimposed relapses and remissions. Onset is typically in late thirties or early forties; initial disease activity is often in spinal cord and not the brain. (d) Secondary progressive MS involves a steady progression of neurological damage with or without superimposed relapses and minor remissions. These MS patients will have previously had relapsing/remitting MS, which could have lasted two to forty years or more.

MS is predominantly a T-lymphocyte mediated disorder, and cytokines may therefore have a key role in the pathogenesis of the disease. MS is the only neurological disorder where therapeutic manipulation of the cytokine system influences development of the disease (4). Thiobarbituric acid reactive substances (TBARS) and F_2-isoprostane levels were shown to be elevated in CSF of MS patients, and HNE was associated with MS lesions, indicative that lipid peroxidation also occurs in MS (57). The presence of oxidized phosphatidylcholine (OxPC) has been demonstrated in MS brain using EO6 monoclonal antibodies (276), supporting evidence that lipid peroxidation is active in this disorder. Although most lesions associated with demyelinating changes in MS are seen in the white matter, recent studies have provided increasing evidence that gray matter is also involved. Neurodegenerative changes have been identified in MS patients, in which substantial neuronal loss is indicated in both cortical gray matter and deep gray matter nuclei. Deep gray matter structures such as the thalamus, putamen, globus pallidus, and caudate have significant roles in processing motor, sensory, cognitive, and emotional information. Neurodegeneration of deep gray matter may correspond to the cognitive impairment commonly seen in MS patients. However, little is known about the pathophysiology of these changes in deep gray matter in MS. A recent study (111) using magnetic field correlation (MFC) imaging indicated increases in iron in the globus pallidus (24%), thalamus (30.6%), and putamen (39.5%) of MS patients. Increased iron deposition in the gray matter correlated positively with total number of MS lesions and showed a modest but significant correlation with neuropsychological tests (111). These findings suggest that abnormal iron deposition has a significant role in the pathophysiology of MS. Antioxidant therapy in multiple sclerosis has been recently reviewed (231).

A recent study showed defective SM metabolism alters normal appearing white matter in MS (376). There is a shift in the lipid composition towards higher phospholipid and lower sphingolipids content in normal appearing white matter and normal appearing gray matter. Modeling studies revealed that the alteration of lipid composition in normal appearing white matter was indicative of elevated repulsive forces between the opposing bilayers that could disrupt the myelin structure. Quantitative analysis of HNE showed accumulation in white matter MS brain tissue. Large accumulation of lysine- and histidine-HNE-adducts was observed in both active and inactive MS. Apart from the activated T lymphocytes, microglia/macrophages that are usually believed to produce detrimental cytokines and ROS; astrocytes also joined the bandwagon in destroying oligodendrocytes in MS. Syncytin (518 amino acid glycoprotein), a placental gene with sequence homology to several retroviral envelope proteins, is identical to the envelope protein of the HERV-W retrovirus. Researchers found that cultured astrocytes which overexpressed syncytin also produced IL-1β, free radicals, and other cytotoxic elements that destroyed myelin (19, 222). Mice injected with a virus that produced syncytin developed movement problems similar to those seen in MS. These MS like symptoms and behavioral deficits were prevented by antioxidant treatment with a polyphenolic agent, ferulic acid. Syncytin interaction with alanine-serine-cysteine transporter-2 (ASCT2) may be a possible mechanism of ROS generation. IL-1β and ROS may contribute to nitric oxide production in astrocytes and further cytokine generation by MS lesions. It is unclear, although both neurons and oligodendrocytes may have low antioxidant defenses, why only oligodendrocytes are targeted by astrocytes and damaged in MS. Manipulation of the syncytin and ASCT2 transporter system may have serious ramifications and should carefully consider their physiologic function.

A. Experimental autoimmune encephalomyelitis (EAE)

EAE is the immune response to immunization with myelin antigens (215) and is an animal model for MS. Recent studies demonstrated a key role for $cPLA_2$ in EAE (215). $cPLA_2$, which can be induced by TNF-α (176), was highly expressed in EAE lesions. Blocking $cPLA_2$ showed a remarkable decrease in both the onset and progression of the disease (215), indicating that $cPLA_2$ has a significant role in both the induction and effector phases of EAE. A second study showed that $cPLA_2$ null mice were resistant to EAE (215). It should be noted that these studies were conducted using C57BL/6 or SV127 mouse strains which are naturally deficient in inflammatory PLA_2/$sPLA_2$ IIA (3). Both MS and EAE are also T helper 1 (Th1) and T helper 17 (Th17) induced autoimmune diseases. Th17 cells produce interleukin-17 (IL-17). A follow-up study by Marusic *et al.* (216) on $cPLA_2\alpha$ deficient mice found significantly attenuated Th1 and interleukin-17 (IL-17) levels compared to wild type. Treatment of EAE rats with $sPLA_2$

inhibitor CHEC-9 (CHEASAAQC) significantly attenuated $sPLA_2$ activity, EAE symptoms, and ED-1 positive microglia/macrophages (74). Recently, MS patients were shown to have elevated $sPLA_2$ activity. These studies suggest that both $cPLA_2$ as well as $sPLA_2$ inhibition may be treatment options for MS.

Data also suggests that oxidative stress plays a major role in MS (117). ROS leading to oxidative stress, generated in excess primarily by macrophages, have been implicated as mediators of axonal damage and demyelination both in MS and EAE model. Different mechanisms have been proposed for how low levels of antioxidants or high levels of ROS can mediate CNS damage in MS. Lower antioxidants may promote elevated LOX activity that triggers the production of leukotrienes, leading to immuno-inflammatory processes in the brain. Excess ROS can stimulate T-cell activity *via* ArAc metabolism or cause BBB dysfunction or demyelination through LPC generated by phospholipases (103, 157). It is routinely accepted that MS pathology affects white matter with axons first undergoing demyelination followed by axonal injury and subsequent neuronal loss. Recent studies challenge this and assert that axonal damage is the critical step in MS pathology that may occur before demyelination and oligodendrocytes become impaired (39). RNS such as peroxynitrite are associated with MS progression and the disease severity is correlated with RNS in the CSF and serum in MS patients. *In vitro* studies provided evidence for differential sensitivity of oligodendrocytes and motor neurons to peroxynitrite.

Mitochondrial uncoupling protein 1 (UCP1) facilitates a proton leak that releases heat from metabolic fuels in brown adipocytes. Homologues of UPC1, UPC2 and UPC3, have been identified in other tissues including immune cells but their role in normal physiology is still enigmatic (56, 175, 219). UCP2 is believed to reduce oxidative stress by attenuating ROS generation during infection and development of atherosclerosis. UCP2-deficient C57BL/6J mice developed significantly higher clinical scores in EAE model. Higher levels of infiltrating T cells were observed in spinal cord along with higher levels of pro-inflammatory cytokines (TNF-α and IL-2) from Th cells as well as increased ROS generation (366). These studies were supported by other groups observing increased NFkB activity in UCP2-deficient mice that lead to increased nitric oxide formation and enhanced inflammatory cytokine generation (23).

XV. Huntington's Disease (HD)

HD is a rare inherited neurological disorder characterized by abnormal body movements and lack of coordination; cognition may also be affected. HD is caused by a trinucleotide repeat expansion in the Huntingtin (*Htt*) gene. The normal gene has fewer than 36 repeats, whereas the mutated *Htt* gene has 40 or more CAG repeats. Since CAG is the codon for glutamine, HD is one of the polyglutamine (polyQ) disorders. HD is associated with upregulated transglutaminase activity (149) in selectively vulnerable brain regions and transglutaminase-catalyzed cross-links co-localize with huntingtin (htt) protein aggregates (240).

Endocannabinoids act as retrograde messengers, and upon release from postsynaptic neurons, regulate further neurotransmitter release by activating presynaptic cannabinoid receptors (78). The endocannabinoid system is *hypoactive* in HD (202), which may underlie the neurotransmission abnormalities of HD and may be the cause of the clinical manifestations of the disease. Inhibition of fatty acid amide hydrolase, monoacylglycerol lipase or the endocannabinoid membrane transporter can enhance endocannabinoid levels and counteract neurochemical deficits and the *hyperkinetic* effects of HD (202).

Endocannabinoids, endogenous agonists of cannabinoid receptors, are comprised of amides, esters, and ethers of long-chain PUFA. *N*-arachidonoylethanolamine (AEA, anandamide) and 2-arachidonylglycerol (2-AG) are well-characterized lipid mediators of the endocannabinoid system (202). The endocannabinoid system has been found to have an important neuroprotective role in CNS injury and neurodegenerative diseases; an extensive review of these studies was recently published (260).

Dietary supplementation with essential fatty acids protected against motor deficits in a transgenic mouse model of HD (69). Ethyl-eicosapentaenoate (Ethyl-EPA, LAX-101 or Miraxion) showed promise in clinical trials and its action is presumed to be through c-Jun amino-terminal kinase (JNK) pathway (275), which belongs to the mitogen activated protein kinase (MAPK) family. There is increasing evidence that JNKs are potent effectors of apoptosis or degeneration in the brain (242). Expression of polyQ-containing proteins has been shown to induce various cellular stress responses including JNK. JNK was shown to be activated in a rat model of HD and contribute to HD neurotoxicity (266).

Minocycline is a semisynthetic tetracycline antibiotic that also has anti-apoptotic properties. Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone, is an essential cofactor in mitochondria electron transport chain where it accepts electrons from complex I and complex II. Combination therapy using minocycline and CoQ_{10} in R6/2 transgenic HD mouse model also provided synergistic benefit (minocycline attenuated microglia proliferation and CoQ_{10} reduced htt protein aggregation) (329). Creatine is a guanidine compound with a key role in energy buffering within the cell. Recent studies showed that combination of CoQ_{10} with creatine provided additive neuroprotective effects in HD models (3-nitropropionic acid in rat and R6/2 transgenic mouse) (393).

A. HD, iron, and lipid peroxidation

In HD, brain iron and ferritin accumulation has been detected in the putamen, caudate nucleus, and globus pallidus. These high iron levels were found early in the disease process and are therefore considered risk factors. While the cause of increased iron in HD is not known, studies have implicated that the huntingtin protein is essential for proper regulation of the iron pathway and an iron response protein. Low serum ferritin levels and elevated ceruloplasmin in HD brains indicate a generalized dysregulation of iron metabolism. A number of studies have focused on the role of impaired energy metabolism and subsequent ROS production in HD. The basal ganglia and cortex of HD patients show decreased levels of glucose and oxygen. Mitochondrial respiration and ATP production are significantly impaired in HD striatal cells. In postmortem brain tissue from HD patients, severe deficiencies of respiratory complexes II and III were found, which are the likely cause of impaired ATP production. The defects in mi-

tochondrial respiration are also a probable cause of increased ROS production. One study in HD reported no increase in 8-OHdG or other markers of DNA oxidation, and no change in lipid peroxidation (16, 213). In other studies, elevated plasma levels of 8-OHdG and HNE have been reported in HD patients. Postmortem striatal and cortex tissue of HD patients showed signs of oxidative damage and increased levels of MDA. Other studies also have shown increases in the lipid peroxidation markers F_2-isoprostane and MDA in HD (213). The evidence for increased ROS in HD suggested that antioxidant defenses might have been upregulated in response. The extent and intensity of HO-1 immunoreactivity were enhanced in HD brain sections and the intensity of staining increased with the severity of the disease. However, little is known about expression of Nrf2 or other Nrf2-regulated proteins in HD brain. In an inducible HD model (inducible expression of exon 1 of the HD gene) in PC12 cell system a number of Nrf2-related genes are upregulated in response to induction of mutant huntingtin (359).

XVI. Amyotrophic Lateral Sclerosis (ALS)

ALS is an adult-onset neurodegenerative disease characterized by progressive loss of spinal cord and cortical motorneurons and is usually fatal within 2–5 years of diagnosis. Approximately 10% of ALS cases are familial (inherited), with the remaining 90% of cases being sporadic in origin (177). Of the familial cases, ~20% (*i.e.*, 2% of all ALS) are due to mutations in the gene for the cytosolic SOD1, which detoxifies superoxide anion radicals to hydrogen peroxide. Expression of a mutant SOD1 protein, with or without residual SOD activity, is necessary to cause ALS phenotype, suggesting a dominant negative mechanism (177). There is strong evidence that the toxicity of mutant SOD1 in ALS is not due to loss of activity, but to the gain of one or more toxic functions that are independent of SOD activity (248). It is believed that mutant SOD1 stimulates oxidative stress and induces mitochondrial dysfunction, excitotoxicity, inflammation, and protein aggregation.

Mammalian cells have evolved a complex physiological response to reduced oxygen availability (hypoxia) in an attempt to prevent energy failure and maintain production of ATP. Many of these physiological responses are regulated by a family of transcription factors denoted hypoxia-inducible factors (HIF), heterodimeric transcription factors consisting of α and β subunits (143, 315). HIF-1β, also known as aryl hydrocarbon receptor nuclear translocator (ARNT) is constitutively expressed (283). Three mammalian α-subunits have been identified, HIF-1α, HIF-2α (EPAS), and HIF-3α, which are all oxygen sensitive but differ in their tissue expression. HIF-1α is also constitutively expressed but is rapidly degraded under normoxic conditions by the ubiquitin–proteosome pathway. HIF-1α stability is regulated by hydroxylation of two proline residues by the oxygen-dependent HIF prolyl-4-hydroxylases. Under hypoxic conditions, HIF-1α is stabilized and hetero-dimerizes with HIF-1β. Thus, HIF-1α serves as the oxygen-sensing subunit of HIF-1 (Fig. 13). HIF-1 binds to hypoxia response element (HRE) in the promoters, upregulating its target genes, including vascular endothelial growth factor (VEGF), glucose transporters, erythropoietin (EPO), and BCL2/adenovirus E1B 19kDa interacting protein 3 (BNIP3). However HIF-1α knockdown did not eliminate VEGF mRNA and had no effect on angiogenesis, suggesting compensatory expression of other angiogenic factors (232, 336). Tumor cell lines lacking HIF-1α had a two-fold increase in expression of interleukin-8 (IL-8) under hypoxic conditions; nuclear factor-κB (NF-κB) appeared to mediate expression of IL-8 in the absence of HIF-1α. HIF-1α knock-down resulted in ROS generation, activation of NFkB, and upregulation of IL-8 (98, 336).

While the factors underlying sporadic ALS remain obscure, an unexpected insight into a possible cause was provided from studies in mice with selective deletion of the HRE element of the promoter of the gene encoding VEGF (68, 256). VEGF is a critical factor controlling growth and permeability of blood vessels in normal development and in response to changing metabolic demands such as hypoxia. Surprisingly, mice with deletion of the HRE element in the VEGF promoter develop profound motor deficits beginning at 5 to 7 months of age. The motor deficits then progress and exhibit all the classical features of ALS: misaccumulated neurofilaments in spinal cord and brainstem motor neurons, degeneration of

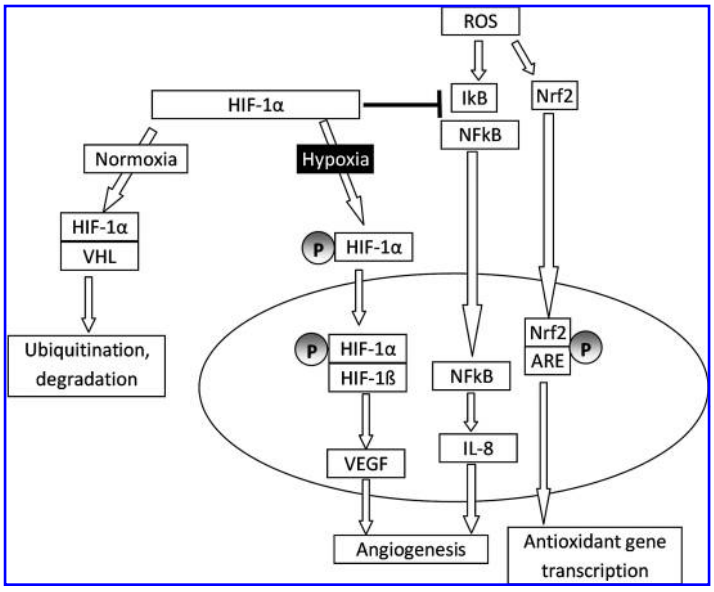


FIG. 13. Under normoxic conditions, hypoxia-inducible factor-1α (HIF-1α) is rapidly degraded by an ubiquitination pathway by the actions of Fe^{2+}, prolylhydroxylases, tumor suppressor protein von Hippel-Lindau protein (VHL). Under hypoxic conditions, HIF-1α stabilizes, gets phosphorylated and translocates to nucleus where it complexes with HIF-1β and activates hypoxia response element (HRE) and initiates gene transcription. Under functional HIF-1α, nuclear factor kB (NFkB) will not be activated and vascular endothelial growth factor (VEGF) solely responsible for angiogenesis. HIF-1α knockout generates ROS and activates NFkB and chemokines such as interleukin-18 (IL-8). ROS also activates Nrf2 thereby initiating antioxidant gene transcription.

motor axons, and muscle atrophy. As in ALS, the neural deficits are selective for motor neurons. One explanation for the specific sensitivity of motor neurons is their high metabolic demand needed to maintain a high rate of neuronal firing. Defects in VEGF regulation could render motor neurons particularly susceptible to hypoxia or vascular insufficiency (323). A recent study provided a link with familial ALS and showed that mutant SOD1 binds to adenine/uridine-rich stability elements in the VEGF 3′-untranslated region (191). ALS-linked SOD1 mutants disrupted the blood–spinal cord barrier in mice by reducing the tight junction proteins zona occludens 1 (ZO1) and causing vascular changes (407).

A role for dysregulated glutamate homeostasis in ALS-mediated neurodegeneration has been established, based on the following evidence: (a) motor neurons showed a marked vulnerability to glutamate excitotoxicity, (b) increased plasma levels of glutamate, decreased glutamate uptake, decreased expression levels of the glial glutamate-transporter EAAT2, and altered glutamine synthetase have been documented in ALS patients, and (c) cerebrospinal fluid (CSF) collected from ALS patients, but not from healthy controls, was excitotoxic in neuronal cultures, which was blocked by glutamate-receptor antagonists. Riluzole (177) (a benzothiazole derivative that acts by reducing glutamate excitotoxicity) remains the only FDA-approved drug for ALS, based on the 3-month improvement in survival observed in two large clinical trials (97).

Targeted expression of SOD1 in neurons or astrocytes of transgenic mice did not lead to ALS, showing that these cell types may not be primarily involved in this disorder (194, 270). It appears likely that a concerted action of several cellular interplayers is a requisite for developing ALS pathology (87). Despite the view of ALS as a motor neuron disorder, new theories have been proposed such as metabolic alterations in this multisystem disorder (86). Studies showed that a significant number of ALS patients present with a stable hypermetabolism that correlated with survival (220). ALS animal model show reduced adiposity and increased rate of energy consumption (87). In these animal models, increased lipid content of the diet extended survival, whereas calorie restriction worsened the symptoms.

A recent review article suggested 24 neuroprotective agents for ALS treatment based on systematic and rational selection process; Taxol also has been suggested to be included in this list (348). Several inflammatory markers such as caspase 1, COX-2, and TNF-α are increased in spinal cord tissue in transgenic mouse models of ALS. Inhibition of COX-2 reduced spinal neurodegeneration and prolonged the survival of ALS transgenic mice (230). The role of COX-2 in ALS as well as the presence of TNF-α suggests that $cPLA_2$ and/or $sPLA_2$ may also be upregulated in ALS to provide ArAc to the COX pathway. TNF-α induces $cPLA_2$, $sPLA_2$ as well as COX-2. This suggests that anti-TNF-α therapy could attenuate the progression of ALS, an option that has not been utilized yet.

A. ALS, iron, and lipid peroxidation

Abnormal increases in iron have been detected at sites of degenerating motor neurons in ALS that can lead to iron-induced lipid peroxidation. Mitochondrial dysfunction (204) and increased cellular uptake of iron due to an increase in lactoferrin have been suggested as possible causes of iron increases in ALS. Defective mitochondrial transport aspects in ALS has been extensively reviewed (204). Increases in oxidative damage to DNA, lipids, and proteins are seen early in the disease process, which may be due, at least in part, to elevated iron levels.

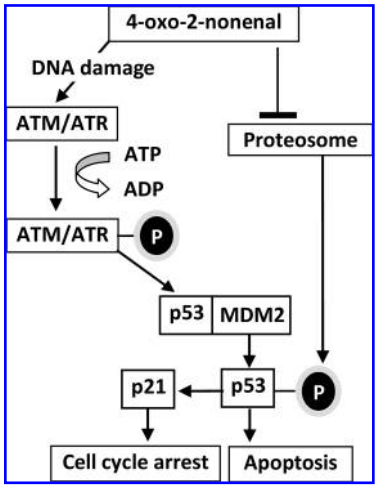


FIG. 14. 4-oxo-2-nonenal (ONE) forms adducts with bases of DNA. The DNA damage triggers autophosphorylation of ATM, which in turn phosphorylates p53. ONE also inhibits proteasome activity, increasing the half-life of p53 and allowing accumulation of ubiquitinated proteins (317). p53 induces apoptosis, or cell cycle arrest by upregulating cyclin-dependent kinase inhibitor p21.

Immunohistochemical studies demonstrated accumulation of the lipid peroxidation adduct 4-oxo-2-nonenal-2′-deoxyguanosine in the spinal cord motor neurons of sporadic ALS patients (317) (Fig. 14). 4-oxo-2-nonenal (ONE) is an aldehyde product originating from peroxidation of ω6 PUFA. In cultured neuroblastoma cells, ONE induced phosphorylation of ataxia telangiectasia-mutated (ATM), which plays an essential role in transmitting DNA damage signals by the phosphorylation of p53. ONE also caused a significant inhibition of proteosome activities, accumulation of ubiquinated proteins, and induced cleavage of poly (ADP-ribose) polymerase (PARP, a hallmark of apoptosis), and apoptosis. Thus ONE activated the p53-dependent apoptosis mechanism *via* activation of p53 signaling and downregulation of p53 turnover. Previously it was thought that HNE was the major lipid hydroperoxide-derived electrophile that reacts with DNA and proteins. It is now recognized that ONE is more reactive than HNE toward DNA bases (192, 317). In the neuroblastoma cell line, HNE failed to induce phosphorylation of ATM and p53, accumulation of ubiquitinated proteins, and PARP cleavage (317). These studies indicated that enhanced lipid peroxidation and formation of ONE, followed by activation of p53 signaling pathway, could be involved in ALS neurodegeneration (Fig. 14). Evidence of increased oxidative DNA damage in ALS was indicated by elevated levels of 8-OHdG in plasma, urine and CSF (213). Several studies have shown increased lipid peroxidation and DNA damage in transgenic mice expressing mutant SOD1 and in neural tissue or sera from ALS patients (13, 321).

XVII. Schizophrenia and Bipolar Disorder

Schizophrenia is marked by disturbances in thinking, emotional reactions, social behavior, with delusions and hal-

lucinations. Drugs that block dopamine receptors alleviate symptoms of schizophrenia, indicative of excess dopaminergic function, while agents that block glutamate receptors induce some of the symptoms of schizophrenia in otherwise normal persons (142).

Recent theories on the neurological deficits of schizophrenia have focused on abnormalities in phospholipid metabolism, particularly increased activity of PLA_2 enzymes and reduced activity of the system that incorporates PUFA into phospholipids (a simultaneous increase in phospholipid hydrolysis and decrease in synthesis) (33, 142). Neither abnormality alone produces schizophrenia but the presence of both does. These abnormalities lead to changes in membrane structure and thus the function of membrane-bound proteins, availability of cell signaling molecules, and the behavior of neurotransmitter systems. This hypothesis is supported by animal studies demonstrating that application of PLA_2 into the brain produces alterations in the dopamine system (142). Also, since phospholipid metabolism has a crucial role in neuronal and synaptic growth and remodeling, it is plausible that defects in this system result in failure of normal neurodevelopment in schizophrenia. There is also evidence that schizophrenia is associated with alterations in lipid transport proteins and membrane phospholipid composition (increase in PS and decrease in PC and PE) (33). Genome studies have found that several genes involved in myelination have decreased expression levels in schizophrenia (33, and reference cited therein).

A number of reports indicate that at least a portion of schizophrenic patients have reduced levels of PUFA, particularly ArAc and DHA, in red cell phospholipids, with low levels particularly associated with negative symptoms (142). ArAc, and the ω-3-fatty acids, DHA and EPA, are important for monoaminergic neurotransmission, brain development, and synaptic functioning (33). This suggests that supplementation with essential fatty acids could alleviate symptoms of schizophrenia. In preliminary studies, however, DHA essentially had no effect and ArAc appeared to worsen symptoms in some schizophrenia patients. Unexpectedly, EPA provided significant improvement, comparable in magnitude to that produced by new atypical antipsychotic drugs, without any of the side effects characteristic of drug treatment. While the efficacy of EPA in treatment of schizophrenia has been reported in several studies (327), its mechanism of action remains obscure, but a possible role of BDNF can be attributed (280, 282).

The combination of EPA and DHA was also beneficial in bipolar disorder (142), which could be due to effects on BDNF/cAMP response element binding protein (CREB)/p38 mitogen-activated protein kinase (MAPK) cascade (280) and/or PLA_2s (186). Decreased DHA and BDNF have been implicated in bipolar disorders (280). BDNF was decreased only in those patients in the late stage of bipolar disorder and were negatively correlated with length of illness (161). Failure of inflammatory defenses in the late stage of the disorder may account for reduction in BDNF and continued elevations in cytokines; thus, these may have the potential to serve as markers of illness progression in bipolar disorder. Dietary deprivation of ω3-PUFA in rats elevated depression levels with corresponding decrease in DHA, expression of BDNF, and activities of CREB and MAPK. Similarly, ω3-enriched fatty acids increased BDNF levels, and enhanced learning and cognitive function after fluid percussion brain injury in rats (118, 385). *In vitro* studies showed DHA addition to astrocyte cultures increased BDNF expression. Dietary deprivation of ω3-PUFA differentially altered the PLA_2 expressions ($cPLA_2$ and $sPLA_2$ mRNA expression and activities were increased, while calcium independent-$iPLA_2$ mRNA expression and activities were decreased) and also increase cyclooxygenase-2 (COX-2) activity and expression (279). The decrease in $iPLA_2$ may prolong the DHA levels in brain but may not be sufficient to arrest their loss. The mood stabilizers such as lithium, carbamazepine, and valproate may interfere with the brain ArAc metabolism (281). Lithium and carbamazepine may selectively affect ArAc selective-$cPLA_2$, whereas valproate may affect acyl-CoA synthetase. These drugs one way or other affect the ArAc cascade (COX-2 and prostaglandin E2) without altering DHA levels. The role of PLA_2 in schizophrenia, how group IIA ($sPLA_2$), group IVA ($cPLA_2$) and group VIA ($iPLA_2$) are associated with brain disorders and conflicting results have been recently reviewed (101, 186).

Alterations in glutamatergic and GABAergic neurotransmitter systems have been implicated in various psychiatric disorders including schizophrenia and bipolar disorder. A number of neurosteroids exhibit the capacity to modulate excitatory and inhibitory neurotransmitter systems in the brain: allopregnanolone is a potent modulator of $GABA_A$ receptors and demonstrates anticonvulsant actions in seizure paradigms. Pregnenolone sulfate and DHEA modulate $GABA_A$ and NMDA receptors. In schizophrenia and bipolar disorder, pregnenolone and DHEA levels were elevated compared to control subjects, while allopregnanolone levels decreased in schizophrenia, suggesting alterations in pregnenolone metabolism. Thus, neurosteroids may be modulators of the pathophysiology of schizophrenia and bipolar disorder, and relevant to treatment of these disorders (217).

There is strong evidence suggesting that oxidative stress may play a role in the pathophysiology of both schizophrenia and bipolar disorder (18, 179). The role of antioxidant defense involving the glutathione system in schizophrenia has been recently reviewed (384). The GSH precursor N-acetyl cysteine improved negative symptoms. Interestingly EPA treatment increased GSH concentrations in medial temporal lobes and correlated with reduction in negative symptoms (34). Damage to the mitochondrial electron transport chain/oxidative stress has been suggested to be an important factor in the pathogenesis of a range of psychiatric disorders (285). Increase in SOD activity in schizophrenia and manic bipolar patients indicate activation of oxidative defense mechanisms in both disorders. This may be due to a compensatory response to the oxidative stress that occurs in acute phase of bipolar episodes. Meta analyses of studies (16 studies; 435 patients and 366 control subjects) in the literature indicated that SOD, catalase, and glutathione peroxidase were not significantly altered in bipolar disorders.

A funnel plot is a useful graph designed to check the existence of publication bias in meta-analyses and is used primarily to detect publication bias (Egger's test). A symmetric inverted funnel shape arises from a 'well-behaved' data set, in which publication bias is unlikely. An asymmetric funnel plot questions the appropriateness of a simple meta-analysis. Eggers's test was positive for SOD, indicative of a publication bias. Kunz *et al.* (179) did not address this issue and certainly further investigation is warranted to sort out the SOD status in these disorders.

It has been suggested that an imbalance between antioxidant enzymes may be a better marker or indication of oxidative stress than their absolute activities. Thus, it was found the SOD/GPx + catalase ratio was increased in manic and depressed but not in euthymic (normal mood) bipolar disorder patients (18). Increase in TBARS suggests increased lipid peroxidation in these disorders. This may be due to significant amount of oxygen consumption to maintain the neuronal membrane potentials during high neuronal activity, which ultimately produce excess ROS. Telomeres are regions of repetitive DNA at the end of chromosomes, which protect the end of the chromosome from destruction. Mitochondrial production of ROS is a key determinant in telomere shortening, considered an indicator of cumulative oxidative stress and a marker of antioxidant defense capacity. Studies have shown that the amount of telomere shortening observed in individuals with chronic bipolar and unipolar disorder represents ~10 years of accelerated aging (18).

XVIII. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent spontaneous seizures due to an imbalance between cerebral excitability and inhibition, with a tendency towards uncontrolled excitability (262). Recurrent severe seizures can lead to death of brain cells. Phenytoin (Dilantin, Phenytek) is a widely used anti-seizure medicine (182). The primary site of action appears to be the motor cortex where spread of seizure activity is inhibited, possibly by promoting sodium efflux from neurons. Phenytoin tends to stabilize the threshold against hyperexcitability caused by excessive stimulation. The current status of new (second generation) antiepileptic drugs has been recently reviewed (38).

The ketogenic diet is an established and effective nonpharmacological symptomatic treatment for epilepsy that has been in clinical use for >80 years (44). The ketogenic diet is a high fat, low protein, and low carbohydrate diet in which >90% of calories are derived from fat, and dietary availability of glucose is minimal. The hallmark feature of the ketogenic diet is production of ketone bodies (β-hydroxybutyrate, acetoacetate, and acetone) in the liver with a concomitant rise in plasma levels. Since glucose (the preferred source of energy, particularly in the brain) is severely restricted, the ketone bodies are used as the energy source in extrahepatic tissues (110). Despite its many years of use, there is still considerable debate over how the ketogenic diet works; several hypotheses have been advanced, but none are widely accepted. One hypothesis, which arrived out of the Epilepsy and Brain Mapping's research, suggests that ketosis, dehydration, and acidosis each appear to play a role, and that there are alterations in (a) acid-base balance, (b) water and electrolyte distribution, (c) lipid concentration, (d) brain energy reserve, or (e) a central action of ketones on the brain. There were attempts to understand how ketogenic diet works in seizure control (148). Animals that were fed ketogenic diet showed twofold increase in hippocampal mitochondrial GSH and GSH/GSSG ratios compared with control diet fed rats. Glutamate cysteine ligase, the rate limiting enzyme in GSH biosynthesis, was increased in rats fed the ketogenic diet. Ketogenic diet decreased H_2O_2 production and mitochondrial DNA damage and improved the mitochondrial redox status.

A recent clinical study in women with epilepsy showed significant elevation of MDA levels (77). In this study of 15 patients, 9 were on monotherapy (using antiepileptic drugs), five were on polytherapy, and one was not taking any antiepileptic drugs. The serum MDA levels in these patients ranged from 1.7 to 2.8 nmol/mL. Women that received sodium valproate showed higher levels of MDA compared to those taking carbamazepine. There were no differences in mean MDA levels (2.13 ± 0.42 nmol/mL) between monotherapy *vs.* polytherapy groups. Valproic acid is a broad spectrum antiepileptic drug used to treat many different types of partial and generalized seizures, as well as bipolar and schizophrenia disorders. It is usually well tolerated although serious complications may occur in some patients due to hepatotoxicity and hyperammonemic encephalopathy due to depletion of carnitine levels. Complete fatty acid oxidation is critical to maintain metabolic homeostasis, especially during periods of fasting and starvation. Mitochondrial β-oxidation of valproic acid involves first its transport within the mitochondria matrix *via* the same pathway as long-chain fatty acids. Valproic acid is first linked to acetyl CoA to form valproyl-CoA, which then crosses the outer mitochondrial membrane. Valproyl-carnitine is then formed. In the mitochondrial matrix, valproyl-carnitine is transformed into valproyl-CoA, which then undergoes β-oxidation (304, 356). Valproic acid toxicities may be due to carnitine depletion and impaired synthesis caused by valproic acid. Carnitine supplementation during valproic acid induced dysfunction of β-oxidation is suggested in high risk patients by several studies (190). On a different note, valproic acid also affected inositol-1-phosphate synthase, inhibited PS synthase, attenuated PI, PS and increased cardiolipin levels (152).

XIX. Conclusions

Recent advances have demonstrated that lipids and their metabolites have broad functions in the CNS as both ligands and substrates for proteins. Lipids alter the geometric properties of membranes and control protein traffic, and provide messenger molecules that mediate communication between cells, suggesting that advances in our understanding of lipid metabolism could have far reaching implications in other genomic, proteomics, and metabolomic fields (95, 268). However, by virtue of their high content of unsaturated fatty acids, lipids are particularly susceptible to oxidation by ROS. Peroxidation of lipids not only alters the membrane structure, but generates toxic by-products that will affect critical protein function; for example, new lipid peroxidation products have been shown to initiate p53-mediated cell death (317). Many studies have demonstrated increased oxidative damage and lipid peroxidation in CNS injuries and neurodegenerative disorders, and increasing evidence points to their role in the pathophysiology of these injuries/disorders. A major focus of current biomedical research is on the substances that are generated from lipids due to oxidative stress in common CNS injuries/disorders. Oxidative stress and lipid oxidation/peroxidation will become an emerging topic that is relevant to human health and disease. The pathophysiological consequence of altered lipid metabolism due to oxidative stress will also play a vital role in determining cell growth, cell death, and cell differentiation. Further research on the oxidized lipid species, the by-products of lipid oxidation/peroxidation, will

undoubtedly provide new and interesting data that will enhance our understanding of their biological functions and contribution to the pathophysiology of CNS pathologies. While animal studies continue to provide mechanistic information and the promise of new therapeutic agents, the true litmus test is demonstration of clinical efficacy. One of the obstacles will be that damage happens very fast after CNS injury, and in neurodegenerative disorders, a large number of neurons have already died by the time clinical symptoms become apparent.

Lipidomic analyses (268, 382) of the lipid oxidative products together with RNA silencing (by selectively silencing lipid metabolizing enzymes in question and in identifying the particular isoforms for the intended CNS injuries/disorders) (126, 255, 317, 349) may provide a powerful tool to elucidate the specific roles of oxidative lipid intermediates in deciding the fate of the neuronal cell. A deeper knowledge of the complexity of lipid signaling will elevate our understanding of the role of lipid metabolism in various CNS disorders, opening new opportunities for drug development and therapies for neurological diseases. As Young *et al.* stated (399) "*our ultimate goal for brain protection in CNS pathologies appears evanescent but it should not be considered as elusive.*"

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Abbreviations Used

Aβ = amyloid beta
AD = Alzheimer's diseases
AEA = *N*-arachidonoylethanolamine; anandamide
2-AG = 2-arachidonylglycerol
ALD = adrenoleukodystrophy
ALS = amyotrophic lateral sclerosis
APP = amyloid precursor protein
ArAc = arachidonic acid
ARE = antioxidant response element
ARNT = aryl hydrocarbon receptor nuclear translocator
ASCT2 = alanine-serine-cysteine transporter-2
ATM = ataxia telangiectasia-mutated
BBB = blood–brain barrier
BDNF = brain-derived neurotrophic factor
BNIP3 = BCL2/adenovirus E1B 19kDa interacting protein 3
CCT = cytidine triphosphate:phosphocholine cytidylyltransferase
CDP-choline = cytidine-5′-diphosphocholine
CEBPβ = CCAAT/enhancer binding protein β
CNS = central nervous system
COMT = catechol-*O*-methyltransferase
CoQ_{10} = Coenzyme Q_{10}
COX/LOX = cyclooxygenase/lipoxygenase
CREB = cAMP response element binding protein
CSF = cerebrospinal fluid
CYP27A1 = sterol 27-hydroxylase
DAG = 1,2-diacylglycerol
DHA = docosahexaenoic acid
DHEA = dehydroepiandrosterone
DMT1 = divalent metal transporter-1
EAE = experimental autoimmune encephalopathy
EIA = enzyme immunoassay
E/L = endosome/lysosome
EPA = ethyl-eicosapentaenoate (Ethyl-EPA, LAX-101 or Miraxion)
EPO = erythropoietin
ER = endoplasmic reticulum
FSE = fast spin echo
GPx = glutathione peroxidases
HD = Huntington's disease
HIF = hypoxia-inducible factor
HMG-CoA = 3-hydroxy-3-methylglutaryl-coenzyme A
HNE = 4-hydroxynonenal
HO-1 = hemeoxygenase-1
HRE = hypoxia response element
ICH = intracerebral hemorrhage
IL-1β = interleukin 1β
INAD = infantile neuroaxonal dystrophy
JNK = c-Jun amino-terminal kinase
Keap1 = Kelch ECH associating protein 1
LBPA = bis(monoacylglycerol)phosphate (lysobisphosphatidic acid)
LPO = lipid peroxidation
Lp-PLA_2 = lipoprotein-PLA_2
L-VSCC = L-type voltage sensitive calcium channels
MCAO = middle cerebral artery occlusion
MDA = malondialdehyde
MMP = matrix metalloprotease
MPTP = 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MRI = magnetic resonance imaging
MS = multiple sclerosis
NBIA = neurodegeneration with brain iron accumulation
NFkB = nuclear factor kB
NPC = Niemann–Pick C disease
NPD1 = neuroprotectin D1
NQO = NADPH:quinone oxidoreductases
Nrf2 = nuclear factor erythroid 2-related factor 2
6-OHDA = 6-hydroxydopamine
8-OHdG = 8-hydroxy-2′-deoxyguanosine
ONE = 4-oxo-2-nonenal
OxPC = oxidized phosphatidylcholine
PAF = platelet activating factor
PARP = poly (ADP-ribose) polymerase
PC = phosphatidylcholine
PE = phosphatidylethanolamine
PI = phosphatidylinositol
PIP_3K = phosphoinositol trisphosphate kinase
PLA_2 = phospholipase A_2
PPAR = peroxisome-proliferator-activated receptor
Prx = peroxiredoxins
PS = phosphatidylserine
PUFA = polyunsaturated fatty acid
RNS = reactive nitrogen species
ROS = reactive oxygen species
RXR = retinoid X receptor
SAH = subarachnoid hemorrhage
SM = sphingomyelin
SMase = sphingomyelinase
sPLA_2 = secretory PLA_2 or inflammatory PLA_2
SREBP = sterol regulatory element binding protein
SSMase = secreted Zn-dependent ASMase
TBARS = thiobarbituric acid reactive substances
Tf = transferring
TfR = transferrin receptor
TNF-α = tumor necrosis factor-α
tPA = tissue plasminogen activator
UCP = uncoupling protein
VEGF = vascular endothelial growth factor
VLCFA = very long chain fatty acids

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