

Significance of Brain Tissue Oxygenation and the Arachidonic Acid Cascade in Stroke

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Abstract

The significance of the hypoxia component of stroke injury is highlighted by hypermetabolic brain tissue enriched with arachidonic acid (AA), a 22:6n-3 polyunsaturated fatty acid. In an ischemic stroke environment in which cerebral blood flow is arrested, oxygen-starved brain tissue initiates the rapid cleavage of AA from the membrane phospholipid bilayer. Once free, AA undergoes both enzyme-independent and enzyme-mediated oxidative metabolism, resulting in the formation of number of biologically active metabolites which themselves contribute to pathological stroke outcomes. This review is intended to examine two divergent roles of molecular dioxygen in brain tissue as (1) a substrate for life-sustaining homeostatic metabolism of glucose and (2) a substrate for pathogenic metabolism of AA under conditions of stroke. Recent developments in research concerning supplemental oxygen therapy as an intervention to correct the hypoxic component of stroke injury are discussed. *Antioxid. Redox Signal.* 14, 1889–1903.

Brain Oxygen Consumption and Regulation

THE HUMAN BRAIN is one of the most metabolically active organs in the body, despite not performing mechanical work like skeletal muscle or the heart. The normal human brain consumes 3.5 ml of O₂ per 100 g of brain tissue per minute, a value which remains constant throughout periods of wakefulness and sleep. This relatively high rate of oxygen consumption is appreciable when compared to the metabolic rate of the entire body. The average man weighs 70 kg and consumes ~250 ml of O₂ per minute in the resting state. With the average human brain weighing 1400 g (~2% of total body weight), it therefore consumes ~49 ml O₂ per minute, or 20% of total body oxygen consumed while at rest (21a, 46, 82) (Table 1). Within the brain, oxygen consumption is highly dynamic and region specific. Gray matter consumes more than twice as much oxygen as white matter, with highest consumption occurring in the medial occipital lobe (125). Oxygen transport from blood to tissue is driven by the oxygen concentration gradient between blood and tissue, moving from the source to the sink. While literature supports capillaries as the principal suppliers of blood to brain tissue, a growing body of evidence suggests that precapillary arterioles also play an active role in oxygen transport (169). Indeed, the major oxygen concentration gradient has been found at the blood–tissue interface of arterioles, not capillaries (38).

At the cellular level, oxygen-sensing systems have evolved to ensure tight regulation of cerebral blood flow and oxygen

homeostasis in the brain to avoid metabolic compromise. The average rate of blood flow in the human brain as a whole is 800 ml/min, or ~15% of the total basal cardiac output. By adjusting vascular tone, brain perfusion is tightly controlled over a wide range of blood pressures, a process termed cerebral autoregulation (2). This powerful autonomic response permits a dynamic flux of cerebral blood flow on the basis of changes in neural activity and metabolic demand within brain regions. This phenomenon is the foundation of functional magnetic resonance imaging (fMRI) which uses blood oxygenation level-dependent (BOLD) contrast imaging to identify changes in the hemodynamic response related to neural activity (73).

When cerebral autoregulation is impaired, or in pathological conditions such as stroke in which cerebral blood flow is disrupted, neuron function is both directly and indirectly regulated in response to brain hypoxia. Direct regulation of neuronal excitation occurs in a hypoxic state by O₂-sensing ion channels that are reversibly inhibited by hypoxia (70). Hypoxia-induced modulation of these channels lead to increased excitability, including “leak” K⁺ currents from TWIK-related acid sensitive (TASK) channels (114). Hypoxia-induced K⁺ leak in TASK channels is an instantaneous open-rectifier current that influences both neuronal resting membrane potential and the duration of action potential (19). In contrast, hypoxia-inducible factor (HIF) transcriptional complex is an indirect regulator of neuronal activity and excitability under hypoxic conditions. Widely conserved among

TABLE 1. OXYGEN CONSUMPTION BY TISSUE

Tissue	O ₂ consumption (ml O ₂ /min/100g)	% of total body O ₂ consumption*
Skin	0.2	5
Resting muscle	1	20
Liver	2	20
Brain	3.5	20
Kidney	5	7.2
Resting heart	8	10–12
Contracting muscle	50	NA

mammalian species and invertebrates alike, the HIF complex exists as a heterodimer composed of constitutively expressed HIF- β and O₂-sensitive HIF- α subunits. Oxygen-dependent enzymatic hydroxylation of proline residues within HIF- α subunits governs protein stability, and transcriptional activity of the HIF complex. More than 70 putative HIF-target genes have been identified to date, expression of which governs an array of vital cellular processes including energy metabolism, angiogenic response, and cell survival (145).

Stroke-Induced Disruption of Cerebral Oxygen Delivery

Today, stroke is the leading cause of long-term disability and the third leading cause of death in the United States, with more than 795,000 Americans afflicted by a new or recurring stroke each year (98). Broadly defined by a cerebrovascular disruption of blood supply to the brain, stroke etiology is classified as either ischemic or hemorrhagic in origin (Fig. 1). Given the hypermetabolic activity of brain tissue and the relatively high oxygen consumption rate, the brain is dependent upon an uninterrupted blood borne supply of substrate for oxidative metabolism. Oxygen is utilized in the brain almost entirely for the oxidation of carbohydrate (21a). Glucose is the preferred metabolic substrate for brain, and without

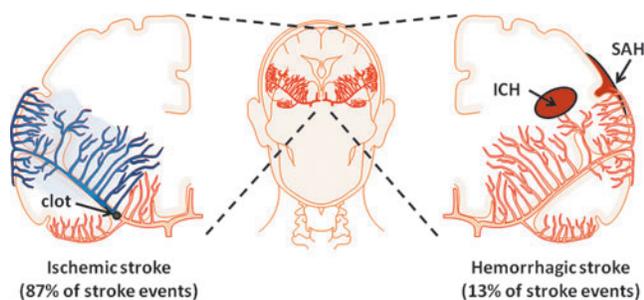


FIG. 1. Stroke etiology. Stroke is a general term used to define pathological conditions in which cerebral blood flow is disrupted. The cause of stroke can be classified as one of two etiological origins: ischemic (*left*) and hemorrhagic (*right*). Ischemic stroke describes the arrest of cerebral blood flow by narrowing and blockage of a cerebral artery (thrombosis) or by a peripheral artery clot (emboli) traveling to and blocking a cerebral artery. Hemorrhagic stroke describes the rupture of a cerebral artery in intracerebral space (ICH) or subarachnoid space (SAH). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

glucose and oxygen stores available to maintain oxidative metabolism, homeostatic brain function is quickly lost. In the case of global cerebral blood flow arrest, consciousness is lost within 10 seconds. The critical level of brain tissue pO_2 beneath which consciousness and normal EEG pattern are lost is 20 mmHg (127). The high oxidative metabolic demands of brain tissue therefore exacerbate the pathological consequences of interrupted circulation of blood to brain tissue as compared to other organs. Irreversible pathological injury to brain tissue occurs within minutes of cerebral blood flow interruption. The adage “time is brain” is commonly expressed to emphasize that human nervous tissue is rapidly and irretrievably lost as the duration of cerebral blood flow disruption progresses. A quantitative assessment of “time is brain” in supratentorial acute ischemic stroke estimated that 1.9 million neurons, and 14 billion synapses are lost per minute due to ischemic cerebrovascular disruption (141).

Ischemic Stroke

The word ischemia originates from the Greek words *ischaimos* which means “to restrain” and *haima* meaning “blood”. In simple terms, ischemic stroke occurs when an artery of the brain is blocked. The brain depends on continuous blood flow to deliver oxygen, nutrients (*i.e.*, glucose, vitamins), and to remove carbon dioxide and cellular waste. When cerebral blood flow is obstructed and brain tissue is no longer able to sustain homeostatic function, the terminal result is energetic failure and cell death. Ischemic stroke has several etiological origins. The most common is due to the narrowing of the arteries in the neck or head. This is most often caused by atherosclerosis—a chronic disease process directly influenced by diet. High fat diets leading to elevated LDL cholesterol and triglyceride levels are significant risk factors for atherogenesis. If cerebrovascular arteries become too narrow, blood cells collect and form blood clots. These blood clots can block the artery where they are formed (thrombosis), or can dislodge and become trapped in smaller or more distant arteries of the brain (embolism).

A thrombotic stroke occurs when diseased or damaged cerebral arteries become blocked by the formation of a blood clot within the brain. Clinically referred to as focal cerebral thrombosis or cerebral infarction, this classification of stroke is responsible for 50% of all clinically presented stroke cases. Cerebral thrombosis can also be divided into an additional two categories that correlate to the location of the blockage within the brain: large vessel thrombosis and small vessel thrombosis. Large vessel thrombosis is the term used when the blockage is in one of the brain’s larger blood-supplying arteries such as the carotid or middle cerebral artery, while small vessel thrombosis involves one (or more) of the brain’s smaller, yet deeper penetrating arteries (Φ :0.2–15 mm). This latter type of stroke is also referred to as a “lacunar” stroke event.

An embolic stroke is also caused by cerebrovascular occlusion, but in this case the clot (or emboli) is formed in peripheral arteries outside of the cerebrovascular system. Often from the heart, these emboli will travel the bloodstream until they become lodged in the brain and cannot travel any further. This naturally restricts the flow of blood to the brain and results in almost immediate physical and neurological deficits. While thrombotic and embolic events are the most

common cause of ischemic stroke, there are many other pathological events, including traumatic injury to the blood vessels of the neck, or disorders of blood clotting, which also induce ischemic stroke of the brain.

Focal Ischemic Stroke and the Ischemic Penumbra

Following focal cerebral ischemia, arrest of blood flow at the site of occlusion produces an ischemic infarct core in which local tissue pO_2 approaches anoxic levels. Liu *et al.* reported that in a rodent model of middle cerebral artery occlusion (MCAO) focal cerebral ischemia rapidly decreased interstitial brain tissue pO_2 to ~4% of baseline at the infarct core as measured by electron paramagnetic resonance (EPR) oximetry (96). Moving away from the ischemic core, cerebral blood perfusion and tissue oxygenation gradually improves (Fig. 2) (96, 133). Still hypoperfused as compared to baseline, neurons in this region of tissue, termed the ischemic penumbra, are still able to preserve ion homeostasis and transmembrane electrical potentials (12, 165). While stringent delineation of the ischemic penumbra is difficult, the most clinically relevant and straightforward definition characterizes it as the ischemic region which, without intervention, would evolve into ischemic infarction over time (175a). This definition, while vague, can be readily applied to characterize the penumbra in a preclinical setting where reproducible stroke-induced infarct is compared across control and stroke intervention groups (133). Additional criteria used to define the ischemic penumbra include site specific cerebral blood flow measurement, documenting diminished protein synthesis in approaching the ischemic core, and spatially resolved mRNA expression analysis of heat-shock protein 70 (hsp70) that is reported to be selectively expressed in the penumbra 3h after permanent MCAO in mice (58, 59, 63).

While rapid restoration of cerebral blood flow at the ischemic site via thrombolytic therapy or mechanical intervention reduces infarct lesion size by salvage of the ischemic penumbra, reperfusion itself also paradoxically contributes to ischemic stroke brain injury. Specifically, reperfusion of cerebral blood flow following ischemia rapidly increases cerebral pO_2 in tissue that is metabolically compromised and highly susceptible to oxidative stress (133). In this state, the

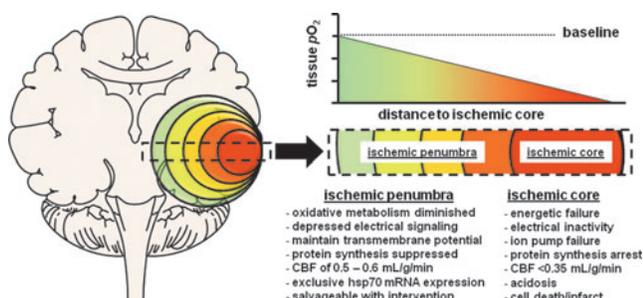


FIG. 2. Characterization of the ischemic penumbra in focal ischemic stroke. In a focal ischemic stroke event, brain tissue oxygenation increases toward baseline (normoxia, green) with distance from the ischemic core (red). Ischemic penumbra and core are delineated by quantitative molecular and physiological differences. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

sudden rise in tissue oxygenation contributes to oxidative damage of brain tissue. Reperfusion also enables peripheral leukocyte recruitment to stroke-affected tissue (124). Leukocytes contribute to stroke pathology by obstructing the microcirculation, disrupting the blood-brain barrier, and infiltrating brain tissue where they release cytokines and propagate inflammation. Furthermore, leukocytes contribute to platelet aggregation in the stroke affected microcirculation causing vasogenic brain edema and hemorrhagic transformation following stroke (135).

Molecular Mechanisms of Ischemic Stroke Pathology

Ischemic stroke-induced hypoxia causes energetic failure (ATP deficiency) due to the lack of oxygen required to maintain cellular respiration. Within 15 minutes of ischemic stroke onset, ATP levels of stroke-affected brain tissue decrease by one-third. As a result, ATP-dependent ion pumps (*i.e.*, Na^+/K^+ ATPase) fail to maintain transmembrane ion gradients, resulting in Ca^{2+} influx through voltage-sensitive Ca^{2+} channels. Ischemia-induced energetic failure in brain tissue also results in excessive extracellular glutamate release. Consequently, glutamate-mediated overstimulation of ionotropic NMDA receptors continues to drive intracellular Ca^{2+} uptake. The subsequent rapid accumulation of intracellular Ca^{2+} results in activation of lipases (*i.e.*, phospholipase A_2) that contribute to membrane degradation and lipid peroxidation in stroke-affected brain tissue enriched with polyunsaturated fatty acids (PUFAs). Moreover, the intracellular Ca^{2+} influx also induces Ca^{2+} and calmodulin co-dependent activation of neuronal and endothelial nitric oxide synthase (NOS). NOS generates nitric oxide (NO) gas, a signaling molecule with well-characterized vasodilatory effects. The potential for NO to improve ischemic stroke-affected cerebral blood flow via vasodilation is clear, however increasing evidence suggests that NO also contributes to oxidative damage during ischemic stroke. With one unpaired electron, NO reacts with most free radicals at near diffusion-limited rates (178). Reaction of NO with superoxide radical (O_2^-) leads to the production of highly reactive peroxynitrite ($ONOO^-$). As much as 2% of oxygen consumed by mitochondria is converted to superoxide anion under normal respiratory conditions (18, 170). In a pathological setting of ischemic stroke, generation of superoxide is greatly enhanced and in combination with elevated NO levels contributes to a massive increase in peroxynitrite formation (154, 155). In addition to evidence supporting peroxynitrite-mediated lipid peroxidation, peroxynitrite also exerts cytotoxic effects directly on mitochondria by inhibiting respiration at complexes I, II, III and V (97, 174).

Hemorrhagic Stroke

Intracerebral hemorrhage (ICH) occurs when a vulnerable blood vessel within the brain bursts, allowing blood to leak inside the brain. The sudden increase in pressure within the brain can cause damage to the brain cells surrounding the blood. If the amount of blood increases rapidly, the sudden buildup in pressure can lead to unconsciousness or death. ICH usually occurs in selective parts of the brain, including the basal ganglia, cerebellum, brainstem, or cortex. The most common cause of ICH is high blood pressure (hypertension). Since high blood pressure itself is asymptomatic, many people

at risk for ICH are not aware that they have high blood pressure, or that it needs to be treated. Less common causes of ICH include trauma, infection, tumors, blood clotting deficiencies, and abnormalities in blood vessels.

Subarachnoid hemorrhage (SAH) occurs when a blood vessel just outside the brain ruptures and the area between the arachnoid membrane and the pia mater rapidly fills with blood. A patient with SAH may have a sudden, intense headache (referred to as a thunderclap headache), neck pain, and nausea or vomiting. SAH may arise spontaneously or due to trauma with the sudden buildup of pressure outside the brain, causing rapid loss of consciousness or death. SAH is most often caused by cerebral aneurysms—small areas of rounded or irregular swellings in the arteries. Where the swelling is most severe, the blood vessel wall becomes weak and prone to rupture.

In addition to a disruption of cerebral perfusion which by itself imposes similar patterns of oxidative stress as compared to ischemic stroke, hemorrhagic stroke also incorporates patterns of oxidative stress associated with iron-mediated free radical injury. Specifically, the rupture of cerebral blood vessels and subsequent red blood cell lysis results in release of heme in hemorrhagic stroke-affected tissue. Heme is metabolized by heme oxygenase (HO) in the brain into iron, carbon monoxide, and biliverdin (64). Following heme degradation by HO, free iron concentration can reach as high as 10 mmol/L, which induces significant brain edema and directly contributes to free radical formation and lipid peroxidation (65). Free iron greatly enhances hydroxyl radical production and subsequent lipid peroxidation via the Fenton reaction (137, 138). Furthermore, as iron deposition in brain tissue increases with age, iron-mediated free radical formation, lipid peroxidation, and neurotoxicity become increasingly relevant in hemorrhagic stroke pathology (158). This is particularly noteworthy given the elevated risk of a cerebral stroke event with age, and the risk for intracranial bleeding in ischemic stroke cases—a phenomenon termed “hemorrhagic transformation” that occurs in as many as 70% of all ischemic stroke patients (13, 90). Finally, carbon monoxide produced by HO-mediated heme metabolism also amplifies generation of reactive oxygen species in brain mitochondria (182), thereby further contributing to elevated oxidative stress under hemorrhagic stroke conditions.

Stroke-Mediated Oxidative Stress in PUFA-Rich Brain

Regardless of stroke mechanism, brain tissue is highly susceptible to oxidative stress as it: a) consumes an inordinate amount of oxygen to meet high metabolic demands, b) contains high concentrations of polyunsaturated fatty acids (PUFAs) that are vulnerable to lipid peroxidation, and c) has lower antioxidant capacity as compared to other organ systems. Neurons are particularly vulnerable to oxidative damage in stroke-affected brain tissue due to lower levels of endogenous antioxidant, glutathione, as compared to resident glial cells (35). The consequence of increased generation of free radical species in stroke-affected brain tissue enriched with PUFAs is an accumulation of lipid peroxidation products that have proven to be culpable neuromodulators of the cell death cascade. Brain tissue is highly enriched with the n-6 PUFA arachidonic acid (AA, 20:4n-6) and the n-3 PUFA docosahexaenoic acid (DHA, 22:6n-3), which are major compo-

nents of phospholipid membranes. Together, AA and DHA account for ~20% of all fatty acids in the mammalian brain (34). Both AA and DHA are nutritionally essential to brain function and structure during early development (26–28, 116, 132, 171), and influence membrane fluidity, signal transduction, and gene transcription throughout life (39, 72, 132, 177). Neither AA nor DHA are synthesized *de novo*, but are obtained from the diet and circulated to the brain directly in the plasma (132), or elongated from n-6 and n-3 PUFA linoleic (18:2n-6) and α -linolenic acid (18:3n-3) precursors in the liver (156). A number of pathological conditions in the human brain are associated with disturbed PUFA metabolism of AA (42, 131), including stroke.

Arachidonic Acid Metabolism in Brain

In resting cells, AA is stored within phospholipid membranes, esterified to glycerol. A receptor-dependent event, requiring a transducing G-protein coupled receptor, initiates phospholipid hydrolysis and releases AA into the intracellular space. Three enzymes mediate this deacylation reaction: phospholipase A₂ (PLA₂), phospholipase C (PLC), and phospholipase D (PLD), each with different sites of attack on the phospholipid backbone. PLA₂ catalyzes the hydrolysis of phospholipids at the sn-2 position, releasing AA in a single-step reaction. By contrast, PLC and PLD do not release free AA directly. Rather, they generate lipid products containing arachidonate (diacylglycerol and phosphatidic acid, respectively), which can be released subsequently by diacylglycerol- and monoacylglycerol-lipases.

Phospholipase A₂

Phospholipase A₂ (PLA₂) isozymes encompass a diverse family of at least 15 different isozyme groups (20) classified into five distinct categories: (a) secreted small molecular weight sPLA₂, (b) larger cytosolic calcium-dependent cPLA₂, (c) calcium-independent iPLA₂, (d) platelet-activating factor acetylhydrolases (PAFA), and (e) lysosomal PLA₂ isozymes. The entire PLA₂ family is characterized by a common function: the enzymatic hydrolysis of the sn-2 ester bond of glycerophospholipids, thereby producing a free fatty acid (*i.e.*, AA) and lysophospholipid (*i.e.*, lysophosphatidylcholine, LPC). Currently, only sPLA₂ and cPLA₂ have well-defined roles in stroke-mediated AA metabolism and are therefore the focus of discussion here.

Under conditions of stroke in which reactive oxygen and nitrogen species are abundant, there occurs a rapid accumulation of free fatty acids, due to increases in intracellular Ca²⁺ and activation of PLA₂s (3, 32, 61, 91, 94, 123, 134, 139, 159, 172). The sPLA₂s are characterized by the requirement of histidine in their active site, Ca²⁺ for catalysis and the presence of six conserved disulfide bonds (20). Under ischemic stroke conditions, sPLA₂ mRNA and protein expression is significantly upregulated (5, 94) and activity is induced by inflammatory cytokine tumor necrosis factor- α (TNF- α) (10). The cPLA₂s are the only PLA₂ which demonstrate a preference for AA in the sn-2 position of phospholipids (21). Localized predominantly in gray matter (160), they lack the disulfide bonding network of sPLA₂s and function through the action of a serine/aspartic acid dyad (20). Following ischemic stroke, cPLA₂ subunit mRNA and protein expression is elevated (123, 159). Recently, a hypoxia-sensitive domain in

the human cPLA₂ promoter region has been characterized, suggesting oxygen-specific transcriptional regulation as well (6). Stroke-induced intracellular Ca²⁺ accumulation mediates cPLA₂ subunit translocation to the membrane phospholipid bilayer (176) and activity is induced by phosphorylation of serine residue 505 by mitogen-activated protein kinase (93, 118, 175). In addition to AA, glycerophospholipid metabolism by PLA₂s following stroke also elevate LPC levels in blood, cerebrospinal fluid, and brain tissue following ischemic and hemorrhagic stroke (61, 62, 79, 164). LPCs act as potent mediators of inflammation in the brain following stroke via stimulated release of interleukin 1 β (162) and subsequent activation of microglia (122, 143).

Once released, free AA has three potential fates: re-incorporation into phospholipids, diffusion outside the cell, and metabolism. In a pathological setting, such as ischemic and hemorrhagic stroke, free AA accumulates and undergoes uncontrolled oxidative metabolism by both enzymatic and nonenzymatic processes (Fig. 3). This uncontrolled metabolism, referred to as the "arachidonic acid cascade", includes the formation of prostaglandins, leukotrienes, thromboxanes, isoprostanes, and nonenzymatic lipid peroxidation products (129). The arachidonic cascade amplifies the overall production of free radicals, both reactive oxygen and nitrogen species, and subsequently oxidative damage to lipids, proteins, and nucleic acids. Taken together, the release of AA by PLA₂ and downstream metabolism plays a significant role in oxidative tissue injury following stroke-induced hypoxia.

Nonenzymatic Oxidative Lipid Metabolism

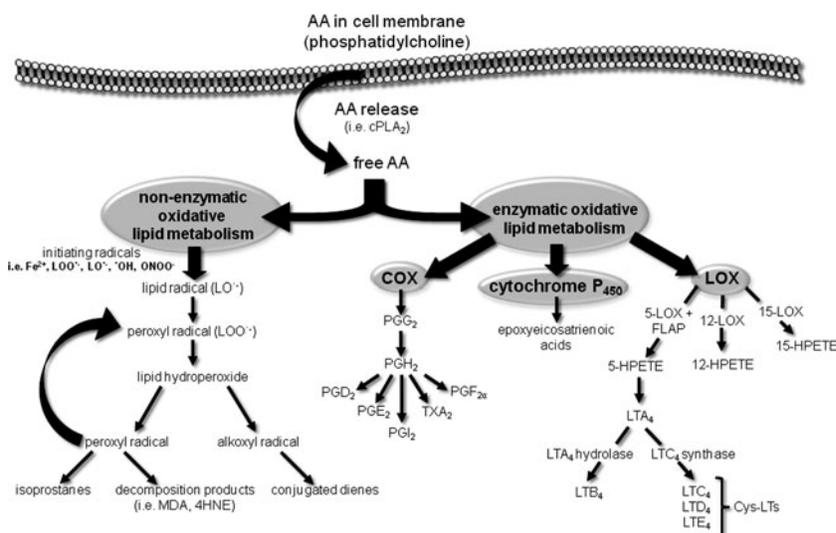
Beyond the initial damage to lipid membranes, reaction of free radical species with double bonds of PUFAs produce alkyl radicals, which in turn react with molecular oxygen to form a peroxy radical (ROO[•]). Peroxy radical can abstract hydrogen from adjacent PUFAs to produce a lipid hydroperoxide (ROOH) and a second alkyl radical, thereby propagating a chain reaction of lipid oxidation (38a). Lipid

peroxides degrade and give rise to α,β -unsaturated aldehydes that include 4-hydroxynonenal (4HNE), malondialdehyde (MDA), and acrolein (4, 40, 76, 126). These aldehydes covalently bind to proteins through reaction with thiol groups and alter their function. They also react with amino groups to form cyclic adducts.

The most well-characterized of the lipid peroxide aldehydes is 4HNE; a nine-carbon α,β -unsaturated aldehyde that at low concentration modulates cellular signaling in brain tissue. Under pathological conditions such as stroke, 4HNE-mediated protein carbonylation induces a number of deleterious effects in cells, including inhibition of nucleic acid synthesis, elevation of intracellular calcium, and inhibition of mitochondrial respiration (43). The localized concentration of 4HNE may increase to as high as 4.5 mM within the phospholipid bilayer, causing protein cross-linking of essential membrane transporters (*i.e.*, glucose and glutamate transporters, sodium/potassium ATPases) (77, 101, 107). The modification of adenine nucleotide translocation by 4HNE also suppresses ADT and ATP transport through the inner mitochondrial phospholipid membrane. Taken together, these actions significantly attenuate the energy-producing capacity of mitochondria in tissue already devastated by stroke-induced energetic failure. The C-3 position of 4HNE is highly reactive: undergoing Michael addition reaction with cellular thiol. This leads to the formation of adducts with endogenous antioxidant glutathione, and ultimately efflux from the cell via glutathione conjugate transporter RLIP76 (51). 4HNE-mediated inactivation of thioredoxin and thioredoxin reductase through modification of cysteine and selenocysteine residues at the active site has also been linked to dysregulation of cellular redox status (41).

Reactive species attack on lipid hydroperoxides also result in the formation of isoprostanes via β -cleavage of the peroxy acid and subsequent molecular rearrangement. Isoprostanes have D-, E-, and F-ringed structures similar to cyclooxygenase-generated prostaglandins, except that their hydrocarbon chains are in the *cis* position in relation to the

FIG. 3. Stroke-induced hypoxia mediates oxidative metabolism of arachidonic acid. Following release from the lipid membrane bi-layer by oxygen-sensitive phospholipase A2 (PLA₂), arachidonic acid (AA) undergoes oxidative metabolism under enzymatic and nonenzymatic mechanisms. FLAP, 5-LOX activating protein; 4HNE, 4-hydroxynonenal; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 12-HPETE, 12-hydroperoxyeicosatetraenoic acid; 15-HPETE, 15-hydroperoxyeicosatetraenoic acid; 5-LOX, 5-lipoxygenase; 12-LOX, 12-lipoxygenase; 15-LOX, 15-lipoxygenase; LTA₄, leukotriene A₄; LTB₄, leukotriene B₄; LTC₄, leukotriene C₄; LTD₄, leukotriene D₄; MDA, malondialdehyde; PGD₂, prostaglandin D₂; PGF_{2a}, prostaglandin F_{2a}; PGG₂, prostaglandin G₂; PGH₂, prostaglandin H₂; PGI₂, prostaglandin I₂; TXA₂, thromboxane A₂.



pentane ring as opposed to the *trans* position observed in prostaglandins. While measurement of isoprostanes is employed as a "gold standard" to quantify cumulative oxidative stress from neurological stress, their bioactivity remains poorly characterized (109, 179). One particular isoprostane metabolite, 15-A_{2t}-IsoP, has been shown to induce neurodegeneration in cultured neurons via mitochondrial ROS production, glutathione depletion, 12-lipoxygenase activation, and caspase cleavage (109). Furthermore, 15-A_{2t}-IsoP has recently been shown to potentiate hypoxia-induced neuronal cell death, suggesting a greater role in stroke pathology (179). Finally, the F₂-isoprostane acts as a potent vasoconstrictor of brain capillaries by inducing COX-mediated synthesis of thromboxane in endothelial cells (88). In a stroke setting, vasoconstriction and platelet aggregation by thromboxane is counterproductive to resolving arteriothrombosis.

Enzymatic Oxidative Lipid Metabolism

Cyclooxygenase, lipoxygenase, and epoxygenase enzymes are pivotal players in the generation of oxygenated derivatives of AA in the pathological setting of stroke. Cyclooxygenases convert AA to prostaglandins and thromboxanes, lipoxygenases catalyze the metabolism of AA into leukotrienes and lipoxins, and epoxygenase activity produces epoxyeicosatrienoic acids. The functional role of these enzymes and downstream lipid-derived products is largely dependent upon environment and cellular localization. In a stroke setting, several products of these pathways act within neurons to modulate the activities of ion channels, protein kinases, ion pumps, and neurotransmitter uptake systems. The newly formed eicosanoids may also exit the cell of origin and act at a distance, by binding to G-protein-coupled receptors present on nearby neurons or glial cells. Finally, the actions of the eicosanoids may be terminated by diffusion, uptake into phospholipids, or enzymatic degradation.

Cyclooxygenase

The cyclooxygenase (COX) family of isozymes is composed of heme-containing bifunctional enzymes with two catalytic sites. The first active site adds two oxygen molecules to AA to form the hydroxy endoperoxide prostaglandin G₂ (PGG₂). Subsequently, PGG₂ translocates to a second active site for peroxidative reduction to prostaglandin H₂ (PGH₂). As there is no channel within the COX monomer to shuttle PGG₂ to the active reducing site, PGG₂ may be reduced on the same or a neighboring enzyme (150). Importantly, the peroxidase activity of COX converts PGG₂ to PGH₂ by removal of oxygen, which may thereby serve as a source of oxygen radicals (86, 153). It has recently been demonstrated that COX-dependent neuronal death is linked to superoxide anion generation (68).

Three cyclooxygenase isoforms (COX-1, COX-2, and COX-3) exist in brain tissue (67). COX-1 and COX-2 are both constitutively expressed in neural tissue (30, 50). COX-1 is traditionally characterized as a "housekeeping" enzyme in homeostatic production of prostaglandins for activities that include maintenance of glycerophospholipid levels and membrane remodeling (37, 103). Unlike COX-1, however, COX-2 expression and activity is induced in response to pathogenic stimuli including ischemic and hemorrhagic stroke (66, 106, 110, 120, 121). Suggestive evidence supports COX-2 isozyme coupling with upstream phospholipase and

downstream synthase activity for the production of lipid signaling eicosanoids. Both cPLA₂ and COX-2 knockout mice share phenotypic similarities and decreased susceptibility to ischemia/reperfusion brain injury (140, 166, 167), supporting a functional coupling between cPLA₂ and COX-2 enzymes (111, 144, 167). While COX-1 and COX-2 are homodimers, sharing 60% homology in cDNA and amino acids, subtle yet significant differences in substrate binding and catalytic sites confer unique properties. COX-1 contains valine residues at positions 434 and 523, while COX-2 possesses isoleucine. This small difference results in a larger and more flexible active site in COX-2 as compared to COX-1; thus accounting for greater eicosanoid production by COX-2 at low AA concentration (87, 129, 152). COX-3 is a recently identified acetaminophen-sensitive COX isoform, highly abundant in astrocytes, endothelial cells, and pericytes, though absent in neurons (29, 81). First described in canines in 2002, COX-3 mRNA is identical to that of COX-1 with the exception that intron 1 is retained. The existence of a COX-1 splice variant that includes intron 1 has also been reported in humans, however, whether this putative human COX-3 encodes a functional COX protein remains questionable and the source of controversy (80).

The COX-mediated metabolism of AA to PGH₂ is a precursor to five primary bioactive prostanoids (PGD₂, PGE₂, PGF_{2α}), thromboxane (TXA₂), and prostacyclin (PGI₂). The highest levels of extracellular prostanoid release in an ischemic stroke setting occurs during reperfusion (161). The prostanoids bind to G protein-coupled receptors that differ in their effects on cAMP and/or phosphoinositol turnover, and intracellular Ca²⁺ mobilization (92). For example, PGE₂ has been shown to elicit both neuroprotective and neurotoxic effects following cerebral ischemia that are dependent on receptor class binding. PGE₂ activation of the E-prostanoid 1 receptor (EP1) disrupts neuronal calcium homeostasis by impairing sodium/calcium exchange, further agonizing Ca²⁺ activation of phospholipase and the arachidonic acid cascade. Pharmacological and gene inhibition of EP1 receptor has been demonstrated to reduce stroke-induced brain injury, oxygen-glucose deprivation, and neurotoxicity (75). Conversely, PGE₂ signaling via EP2 receptor is neuroprotective in cerebral ischemia and dependent on activation of the cAMP-PKA pathway (102). Such paradoxical outcomes suggest an additional level of complexity in the prostanoid response to ischemic injury that warrants further investigation.

Lipoxygenases

The first human lipoxygenase (LOX) activity was described in platelets via the transformation of AA to a prostaglandin-like endoperoxide (54). Today, the LOX enzyme family includes four members that are classified on the basis of the carbon position in which they oxidize arachidonic acid: 5-, 8-, 12-, and 15-LOX. Despite variances in amino acid sequences and tissue distribution amongst family members, the active site of each isozyme is highly conserved (11). The structure of LOX at the active site is composed of an N-terminal beta-barrel domain and a C-terminal domain containing a hydrophobic substrate-binding site (49). A non-heme iron atom is coordinated by three histidine residues and the carboxy-terminal isoleucine. The oxidation of ferrous iron (Fe²⁺) to ferric iron (Fe³⁺) activates the enzyme that is then capable of excising a hydrogen atom from a hydrocarbon at one of the

four double bonds. This abstraction generates a radical metabolite of AA that rapidly reorganizes its double bonds to take on a more stable conformation. Next, insertion of molecular oxygen generates a hydroperoxide radical which is reduced to the hydroperoxide anion by the simultaneous oxidation of iron to the ferric state (49). A proton is then accepted to form a highly reactive fatty acid hydroperoxide; hydroperoxyeicosatetraenoic acid (HPETE). These hydroperoxide derivatives of AA are short-lived, and readily metabolized into more stable compounds, including hydroxyeicosatetraenoic acids (HETEs) and leukotrienes.

While each LOX isoform carries out the same general reaction, namely hydrogen abstraction from arachidonic acid, each has a unique gene structure, amino acid sequence, and tissue distribution profile. Only three forms of LOX are present in brain tissue, 5-, 12-, and 15-LOX (129). Of these, 12-LOX is the most abundant isoform found in the brain (55), with significant mRNA expression in rat cortical neurons, astrocytes, and oligodendrocytes (16). 5-LOX activity requires a small 18 kDa protein known as FLAP (5-LOX activating protein) for leukotriene synthesis (22). Evidence suggests that FLAP binds AA for presentation to 5-LOX (1, 100). The LOX-mediated metabolites of AA serve as second messengers following stroke by modulating inflammation, apoptosis, and synaptic activity.

Hydroperoxy- and Hydroxy- Eicosatetraenoic Acids

While short-lived, HPETEs are potent neurotoxins. Highly reactive oxygen radicals are produced during the conversion of HPETEs to HETEs (74), contributing to the overall burden of oxidative stress following stroke. Under conditions of glutathione (GSH) depletion, as in acute focal stroke, 12-LOX derived 12-HPETE triggers nitric oxide (NO) induced neural cell death (24). Meanwhile, inhibitors of cPLA₂ and 12-LOX have been demonstrated to prevent neurotoxicity *in vitro* (84). Recent evidence supports that the hydroxy- (HETE) derivatives of LOX-mediated metabolism also possess potent biological activity as well. 12-HETE has been shown to increase mitochondrial NO production, to induce cytochrome C release, and subsequently cause mitochondrial dysfunction (112). 5-, 12-, and 15-HETE are potent mediators of increased vascular permeability (57). Elevated levels of HETEs in a setting of ischemic and hemorrhagic stroke may therefore contribute to the documented phenomenon of blood brain barrier breakdown and stroke-induced edema (71).

Leukotrienes

The 5-LOX catalyzed dehydration reaction generates an epoxide intermediate, leukotriene A₄ (LTA₄) (129). LTA₄ is a highly unstable intermediate that is readily hydrolyzed to LTB₄ or conjugated with glutathione by cysteinyl leukotriene C₄ synthase to produce leukotriene C₄ (LTC₄), leukotriene D₄ (LTD₄), and leukotriene E₄ (LTE₄) (147). Together, LTC₄, LTD₄, and LTE₄ are collectively referred to as cysteinyl-leukotrienes (Cys-LTs). Total leukotriene levels accumulate in the mammalian brain and cerebral spinal fluid during and after cerebral ischemia (130, 173). LTB₄ is characterized as a powerful chemotactic agent that induces adhesion of pro-inflammatory leukocytes to the endothelium; stimulates phagocytosis, and activates neutrophils and other leukocytes in a paracrine manner (25, 33, 44, 146). The Cys-LTs are potent

vasoconstrictors of both venous and arterial smooth muscle (105, 142), and are also reported to produce vascular leak and vasogenic edema (36). Furthermore, pharmacological inhibition of Cys-LT receptors attenuates pathological outcomes in a rodent model of focal cerebral ischemia, suggesting roles for Cys-LTs in mediating ischemic injury (31, 44). Like LTB₄, Cys-LTs also operate as potent activators and chemoattractants for leukocytes, particularly eosinophils and monocytes, thereby indirectly propagating inflammation and oxidative stress (44).

Cytochrome P₄₅₀

Cytochrome P₄₅₀ epoxygenases (cP₄₅₀) are heme-containing enzymes that use molecular oxygen to oxidize PUFAs by transfer of one atom of oxygen to their substrate and the other to water. Brain cP₄₅₀ epoxygenases metabolize AA by inserting a single oxygen atom into an unactivated hydrocarbon at one of the four double bonds; thereby producing four regioisomers: 5,6-, 8,9-, 11,12-, and 14,15-epoxyeicosatrienoic acids (EETs). Compared to other organs, expression of cP₄₅₀ in brain tissue is quite low, accounting for only 1%–10% of levels observed in liver (60, 163). Relative expression of cP₄₅₀ in brain tissue is found to be highest in astrocytes localized near blood vessels, as well as the adjacent vascular endothelium (128). The activity of cP₄₅₀ epoxygenases localized in and around the cerebrovasculature has been shown to modulate angiogenesis via capillary endothelial migration and tube formation (104, 108). In a pathological setting, such as ischemic stroke, EETs also serve as potent vasodilators of cerebral arterioles by increasing K⁺ currents in cerebral arteriole smooth muscle cells (47). Elevated extracellular glutamate, as released by neurons and astrocytes under conditions of acute ischemic stroke, increases the formation of EETs in cultured astrocytes (8). Importantly, glutamate alone has no effect on isolated cerebral blood vessels (56, 168). However, when injected into rat brain tissue via cranial window, glutamate induces significant dilation of pial arterioles (8). A cP₄₅₀ inhibitor, miconazole, abrogates this effect (7, 8), suggesting that glutamate-stimulated activity of cP₄₅₀ enhances EET formation and vasodilation.

In addition to their vasodilatory effects, beneficial roles of EETs have been demonstrated within the vasculature that are independent of smooth muscle cell relaxation (85). Specifically, it has been shown that EETs possess potent anti-inflammatory effects by inhibiting cytokine-induced endothelial cell adhesion molecule expression and preventing leukocyte adhesion to the vascular wall (23, 119, 181). More recently, EETs have been shown to protect neurons (83) and astrocytes (95) against ischemic cell death induced *in vitro* by oxygen–glucose deprivation. Like other eicosanoids, the biological activity of EETs is expected to occur via cell surface receptors. To date, however, the identification of putative EET receptors and mechanisms of protection remain to be adequately characterized (85).

Once metabolized by cP₄₅₀, AA-derived EETs are subject to several metabolic fates, including incorporation back into membrane phospholipids, and hydration by soluble epoxide hydrolase (sEH) (180) to di-hydroxyeicosatrienoic acids (DHET). No essential function of DHETs has yet to be identified (157). Strategies to inhibit sEH activity in order to prolong the neuroprotective action of EETs are currently of

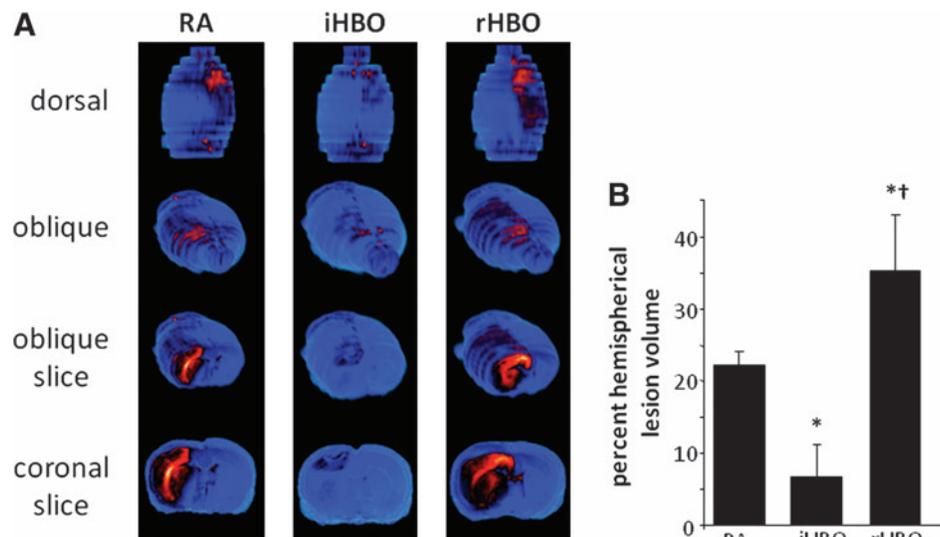


FIG. 4. Hyperbaric oxygen therapy reduces during, exacerbates at reperfusion, acute ischemic stroke infarct volume. (A) Representative T2-weighted MR images were acquired 48 h after MCAO in rodents kept in room air during ischemia (RA, control), or receiving hyperbaric oxygen (100% O₂ at 2 ATA) during ischemia (iHBO) or immediately following reperfusion (rHBO). Color look up table applied—shift from blue to red denotes edema and stroke-induced infarct. Three-dimensional reconstruction of coronal slices permits visualization of the brain from dorsal and oblique positions. HBO during MCAO significantly decreased stroke-induced lesion volume in iHBO animals, while in rHBO animals is increased lesion volume. (B) Percent hemispherical lesion volume corrected for edema >5 * $p < 0.05$ vs RA, † $p < 0.05$ vs iHBO. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

therapeutic interest. Inhibitors of sEH have historically been developed as anti-hypertensive agents, but recent data indicate they also decrease vascular smooth muscle proliferation, prevent cardiac hypertrophy, and improve renal hemodynamics (157).

Supplemental Oxygen Therapy in Focal Ischemic Stroke

In 1662, an English physician and clergyman named Henshaw was the first to use compressed air controlled by large organ bellows to manipulate the atmospheric pressure in a sealed room. He suggested that “in times of good health this domicilium is proposed as a good expedient to help digestion, to promote insensible respiration, and consequently, of excellent use for the prevention of most affections at the lungs” (31a). Two centuries later, a French physician, Junod, was the first to commission the construction of a hyperbaric chamber dedicated for medical use (151). While the purported benefits of early hyperbaric chambers were anecdotal, they laid the foundation for rigorous scientific development. In 1960, Dutch surgeon and engineer Ite Boerema published groundbreaking research demonstrating the capacity for hyperbaric oxygen (HBO)-induced plasma-dissolved oxygen to sustain life in exsanguinated pigs (17). Five years after Boerema’s pivotal discovery, Swedish physicians Ingvar and Lassen reported improved outcomes in four stroke patients treated with HBO—reversing neurological deficits and electroencephalography abnormalities (69).

Since then, a number of small animal and clinical case reports have demonstrated both efficacious and deleterious outcomes for HBO and normobaric oxygen (100% O₂, 1 ATA, NBO) in the treatment of AIS. Three clinical pilot studies to probe the efficacy of HBO to treat acute ischemic stroke reported mixed and potentially harmful outcomes with HBO treatments that overlapped thrombolytic therapy or were applied as late as 2 weeks after the onset of stroke (9, 117, 136). Indeed, preclinical investigation by our laboratory has un-

covered specific application phases during ischemia and following reperfusion in which HBO mediates both protective and harmful outcomes (Fig. 4) (133). HBO applied during MCAO-induced ischemia was found to correct the hypoxia component of stroke injury sufficiently. Mice were selectively implanted with oxygen-sensitive EPR probe in cortical tissue of the ischemic penumbra (Fig. 5). This region was identified as ischemic penumbra as it was spared from stroke-induced lesion when HBO was applied during ischemia. In addition to reduction of stroke lesion volume, rodents receiving HBO during ischemia had significantly attenuated markers of oxidative stress (*i.e.*, 4HNE) in the penumbra region (133). Conversely, HBO at the onset of ischemic stroke reperfusion, when ROS are known to be elevated (78), exacerbated oxidative stress while worsening stroke outcomes. Others have reported a limited window between 3 h and 6 h acute ischemic stroke reperfusion in which HBO is beneficial, but harmful at 12 h and beyond (14, 99). Given the distinct and limited window of HBO therapy, the challenge now comes in translating these findings to an efficient and effective clinical response. Improved diagnostic processes to rapidly and repeatedly monitor cerebral perfusion of acute ischemic stroke patients would benefit the clinical prescription of HBO therapy.

Conclusions

When one considers the unique physiological and biochemical properties of hypermetabolic brain tissue, with a voracious appetite for oxygen in an environment enriched with oxidation-prone PUFAs such as arachidonic acid, the dire physiological consequences of stroke-induced hypoxia is readily apparent. Preclinical models of stroke have revealed substantive evidence of elevated oxidants as early as 20 minutes following the onset of stroke (89, 113), and uncontrolled oxidative metabolism of AA as early as 1 hour following hemorrhagic and ischemic stroke (45, 148). In the case

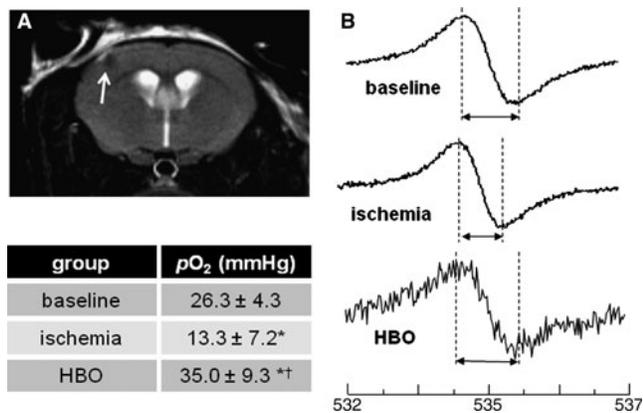


FIG. 5. HBO corrects stroke-induced hypoxia during middle cerebral artery occlusion in rodents. (A) Oxygen-sensitive EPR oximetry probe was implanted in the ischemic penumbra of rodents in the week prior to MCAO using stereotaxic coordinates -0.1 mm bregma, $+2.0$ mm lateral, -1.0 mm dorsal. Placement of the probe was verified using MRI. (B) EPR spectra were acquired prior to MCAO to characterize normal cortical pO_2 (baseline). After MCAO-induced focal ischemia, brain pO_2 in the ischemic penumbra significantly dropped to $\sim 50\%$ of baseline level. Correction of stroke-induced hypoxia was achieved with HBO during ischemia in the penumbra zone. Increased noise in EPR signal was encountered in the HBO group due to greater distance between probe and resonator as a result of the hyperbaric chamber. Representative EPR spectra shown. $N \geq 3$, * $p < 0.05$ vs baseline, † $p < 0.05$ vs ischemia.

of ischemic stroke, a significant increase in reactive oxygen species and AA metabolism accompany reoxygenation of brain tissue following reperfusion of stroke-affected tissue (52). The rapid and overwhelming imbalance of pro-oxidants over antioxidants in stroke injury leads to a pathological setting that is highly resistant to late phase (>6 h after stroke onset) therapeutic intervention. Potential neuroprotective agents administered after the onset of stroke that aim to salvage ischemic tissue, limit infarct size, and resolve stroke-mediated oxidative stress and inflammation have failed in clinical studies. Of 114 neuroprotective agents tested during the 20th century, none were proven successful in clinical trials (48). As clinicians and scientists recognize the significant contribution of the hypoxia component of stroke injury to the limited therapeutic window for intervention, research emphasis is shifting from late phase treatment modalities toward acute phase (<6 hours) and prophylactic treatment regimens.

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Abbreviations Used

4HNE = 4-hydroxynonenal
 AA = arachidonic acid
 AIS = acute ischemic stroke
 BOLD = blood oxygenation level-dependent
 COX = cyclooxygenase
 cP₄₅₀ = cytochrome P₄₅₀
 cPLA₂ = cytosolic calcium dependent
 phospholipase A₂
 Cys-LTs = cysteinyl-leukotrienes
 DHA = docosahexaenoic acid
 DHET = dihydroxyeicosatrienoic acid
 EETs = epoxyeicosatrienoic acids
 EP1 = E-prostanoid 1 receptor
 EPR = electron paramagnetic resonance
 Fe²⁺ = ferrous iron
 Fe³⁺ = ferric iron
 FLAP = 5-LOX activating protein
 fMRI = functional magnetic resonance imaging
 HBO = hyperbaric oxygen
 HETE = hydroxyeicosatetraenoic acid
 HIF = hypoxia-inducible factor
 HO = heme oxygenase
 HPETE = hydroperoxyeicosatetraenoic acid
 ICH = intracerebral hemorrhage
 iHBO = hyperbaric oxygen during ischemia
 iPLA₂ = calcium independent phospholipase A₂
 LDL = low density lipoprotein
 LOX = lipoxygenase
 LPC = lysophosphatidylcholine

LTA₄ = leukotriene A₄
 LTB₄ = leukotriene B₄
 LTC₄ = leukotriene C₄
 LTD₄ = leukotriene D₄
 LTE₄ = leukotriene E₄
 MCAO = middle cerebral artery occlusion
 MDA = malondialdehyde
 NO = nitric oxide
 NOS = nitric oxide synthase
 O₂⁻ = superoxide radical
 ONOO⁻ = peroxynitrite
 PAFA = platelet-activating factor acetylhydrolase
 PGF_{2a} = prostaglandin F_{2a}
 PGG₂ = hydroxy endoperoxide prostaglandin G₂
 PGH₂ = prostaglandin H₂
 PGI₂ = prostacyclin I
 PLA₂ = phospholipase A₂
 PLC = phospholipase C
 PLD = phospholipase D
 PUFA = polyunsaturated fatty acid
 rHBO = hyperbaric oxygen after reperfusion
 ROO⁻ = peroxy radical
 ROOH = lipid hydroperoxide
 ROS = reactive oxygen species
 SAH = subarachnoid hemorrhage
 sHE = soluble epoxide hydrolase
 sPLA₂ = secreted small molecular weight
 phospholipase A₂
 TASK = TWIK-related acid sensitive channel
 TNF- α = tumor necrosis factor alpha
 TXA₂ = thromboxane A₂

