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Cyclooxygenase- and cytochrome P450-derived eicosanoids in stroke

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Abstract

Arachidonic acid (AA) is metabolized by cyclooxygenase (COX) and cytochrome P450 (CYP) enzymes into eicosanoids, which are involved in cardiovascular diseases and stroke. Evidence has demonstrated the important functions of these eicosanoids in regulating cerebral vascular tone, cerebral blood flow, and autoregulation of cerebral circulation. Although COX-2 inhibitors have been suggested as potential treatments for stroke, adverse events, including an increased risk of stroke, occur following long-term use of coxibs. It is important to note that prolonged treatment with rofecoxib increased circulating levels of 20-hydroxyeicosatetraenoic acid (20-HETE), and 20-HETE blockade is a possible strategy to prevent coxib-induced stroke events. It appears that 20-HETE has detrimental effects in the brain, and that its blockade exerts cerebroprotection against ischemic stroke and subarachnoid hemorrhage (SAH). There is clear evidence that activation of EP2 and EP4 receptors exerts cerebroprotection against ischemic stroke. Several elegant studies have contributed to defining the importance of stabilizing the levels of epoxyeicosatrienoic acids (EETs), by inhibiting or deleting soluble epoxide hydrolase (sEH), in stroke research. These reports support the notion that sEH blockade is cerebroprotective against ischemic stroke and SAH. Here, we summarize recent findings implicating these eicosanoid pathways in cerebral vascular function and stroke. We also discuss the development of animal models with targeted gene deletion and specific enzymatic inhibitors in each pathway to identify potential targets for the treatment of ischemic stroke and SAH.

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Conflict of Interest

There are no conflicts to declare.

Keywords

Cyclooxygenase; cytochrome P450; eicosanoids; soluble epoxide hydrolase; stroke

1. Introduction

Stroke, the fifth leading cause of death, is one of the most life-threatening cerebrovascular disorders in the U.S. [1, 2]. According to a 2015 report from the CDC, every year more than 795,000 people in the U.S. have a stroke. Also, strokes kill almost 130,000 Americans each year, accounting for about 1 out of every 20 deaths. There are two main types of stroke: ischemic and hemorrhagic. It has been estimated that ischemic stroke accounts for about 80%–85% of all stroke incidents, while hemorrhagic stroke accounts for the remaining 15%–20% of stroke incidents [1, 2]. Ischemic stroke occurs when a thrombus or embolus blocks cerebrovascular circulation, resulting in irreversible damage to the ischemic core and its surrounding region. Hemorrhagic stroke is mainly due to the rupture of cerebral aneurysms, resulting in subarachnoid hemorrhage (SAH) and intracranial hemorrhage [3].

The use of recombinant tissue plasminogen activator (rtPA) has been the standard of care for treatment of acute ischemic stroke. However, patients with ischemic stroke need to receive this drug within therapeutic window of four-and-a-half hours. Also, there is increasing concern that treatment of rtPA may cause side effects, including the disruption of the blood brain barrier, as well as seizures and progressive neuronal damage. Although rtPA treatment provides significant benefits for stroke patients, this therapy did not show significant benefits for patients with large artery occlusions [4]. To resolve this issue, five clinical trials, including MR CLEAN, ESCAPE, EXTEND IA, SWIFT PRIME, and REVASCAT, were conducted to evaluate the use of endovascular thrombectomy in stroke patients [4, 5]. The results from these clinical trials show that this therapy provides consistent benefits for stroke patients even when it was performed beyond 4.5 h in patients who had already received rtPA [5, 6]. Thus, although more clinical studies are needed to validate the outcomes of these studies, endovascular thrombectomy could be a promising therapy for the treatment of ischemic stroke in the near future. Although there have been significant advances in understanding the pathophysiology following stroke, current treatments for stroke are limited in both their utility (e.g., rtPA) and effectiveness (e.g., aspirin). Therefore, there is a critical need for basic and clinical research to investigate potential therapeutic targets for the treatment of stroke.

Since cerebral vascular function and dysfunction are the key factors in the onset and progression of stroke, this review aims to provide important information about how cyclooxygenase (COX) and cytochrome P450 (CYP)- derived eicosanoids affect cerebral vascular function, as well as the role of these lipid effector molecules in ischemic stroke and SAH.

2. Regulation of cerebral vascular function by COX- and CYP-eicosanoids

2.1. Role of COX-eicosanoids in cerebral vascular function

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid that is usually esterified to the second carbon in membrane phospholipids. The release of AA from phospholipids is achieved through the activity of phospholipase A_2 (PLA_2), which specifically recognizes the *sn*-2 acyl bond of phospholipids and catalytically hydrolyzes the bond releasing AA and lysophospholipids. AA is metabolized to prostaglandin H_2 (PGH_2) by COX-1 or COX-2. COX-1 is constitutively expressed in most cells and is involved in normal physiologic functions [7]. COX-2 is expressed in many organs, such as the brain, and it is highly inducible by pro-inflammatory cytokines [7]. PGH_2 is the substrate for the activities of tissue-specific isomerases and synthases that synthesize PG_2 and TX (Figure 1) [8]. The major PGs are PGD_2, PGE_2, PGI_2, and $PGF_{2\alpha}$; the major TX is TXA_2. The action of these PGs and TXA_2 is mediated through the binding of these products into their membrane-bound receptors, including DP, EPs (EP_1 to EP_4), IP, FP, and TP receptors (Figure 1) [9, 10]. For an overview and function of COXs, there are several excellent reviews regarding to COX-1 and inducible COX-2 in the brain [11–13].

In the cerebral vascular system, COX-1 and COX-2 are important in the modulation of cerebral blood flow [14]. For example, indomethacin, a nonselective inhibitor of COX-1 and COX-2, reduced resting cerebral blood flow and attenuated elevations in cerebral blood flow produced by endothelium-dependent vasodilators [15, 16]. However, previous studies [17, 18] demonstrated that indomethacin has off-target effects that are unrelated to COX inhibition, including inhibition of IP receptor and cAMP-dependent protein kinase activity. Interestingly, Niwa *et al.* [19] have found that COX-1 knockout (KO) and SC-560 (a selective COX-1 inhibitor) significantly attenuated resting cerebral blood flow by 13% to 20%, respectively. Further investigation showed that SC-560 attenuated the cerebral blood flow induced by hypercapnia, bradykinin, calcium ionophore A23187, and AA in wild-type mice but not COX-1 KO mice [19]. These findings demonstrate that COX-1 has a critical role in maintaining resting vascular tone and in selective vasodilator responses in cerebral circulation. To determine the importance of COX-2 in cerebral circulation, Niwa *et al.* [20] showed that NS-398, a selective inhibitor of COX-2, attenuated the increase of somatosensory cortex blood flow induced by vibrissal stimulation. However, neither NS-398 nor COX-2 KO affected increases in cerebral blood flow induced by hypercapnia, acetylcholine, or bradykinin. These results provide solid evidence that COX-2 is important to increase cortex blood flow that accompanies neural activity.

2.2. Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in cerebral vascular function

20-HETE, the ω-hydroxylation product of AA, is the principal AA metabolite of CYP enzymes in vascular smooth muscle [21] and kidney [22]. Synthesis of 20-HETE is catalyzed by the CYP4A gene family [21] (Figure 2). This subfamily encodes several CYP enzymes in different species. In the rat, four CYP4A enzymes have been identified: CYP4A1, CYP4A2, CYP4A3, and CYP4A8 [23]. These isoforms, although sharing 66%–98% homology and common catalytic activity, are expressed in the liver, kidney, and brain [24]. The recombinant CYP4A1, CYP4A2, and CYP4A3, but not CYP4A8, catalyzed AA

ω-hydroxylation to 20-HETE with the highest catalytic efficiency (V_{max}/K_m) for CYP4A1, followed by CYP4A2 and CYP4A3 [25]. In the mouse, four Cyp4a enzymes have been identified: Cyp4a10, Cyp4a12a, Cyp4a12b, and Cyp4a14. Muller *et al.* [26] have demonstrated that AA ω-hydroxylation is catalyzed by Cyp4a10, Cyp4a12a, and Cyp4a12b. Cyp4a12a and Cyp4a12b have similar catalytic activity for 20-HETE production, with a V_{max} value of about 10/min and a K_m value of about 20–40 μM [26]. The ω-hydroxylase activity of AA for Cyp4a10 is about 25 to 75-fold lower than that of Cyp4a12 isoforms. These results suggest that Cyp4a12 isoforms constitute the major source of 20-HETE synthesis. Notably, besides CYP4A enzymes, CYP4F isoforms are also important for 20-HETE production [27].

In the cerebral vascular system, 20-HETE production was first identified in 1994 [28] and a study by Gebremedhin *et al.* [29] has demonstrated that CYP4A1, CYP4A2, CYP4A3, and CYP4A8 are expressed in rat cerebral microvessels. 20-HETE is a potent vasoconstrictor that depolarizes vascular smooth muscle cells by inhibiting K^+ channel activity and is important in regulating renal hemodynamics and renal function [30]. In cerebral microcirculation, Gebremedhin *et al.* [29] showed that an elevation in transmural pressure, from 20 to 140 mm Hg, increased 20-HETE levels by 6-fold, which was determined by GC/MS, in cerebral arteries. Moreover, they also showed that 20-HETE blockade by DDMS and 20-HETE antagonists attenuated autoregulation of CBF to elevations of arterial pressure [29]. In pressurized cerebral arterial segments, 20-HETE elicits vascular contraction that is triggered by inhibition of the activity of the large conductance Ca^{2+}-activated K^+ channel (K_{ca}) and increasing influx of Ca^{2+} through the activation of L-type Ca^{2+} channels [28]. Taken together, these results support the notion that 20-HETE has vital function in autoregulation of cerebral blood flow.

2.3 Role of epoxyeicosatrienoic acids (EETs) in cerebral vascular function

AA is epoxidized by the CYP enzymes into four epoxyeicosatrienoic acids, 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET (Figure 2). EETs are further metabolized by soluble epoxide hydrolase (sEH) to the corresponding vic-dihydroxyeicosatrienoic acids (DHETs) (Figure 2) [31]. EETs production has been attributed to the CYP 2C and 2J isoforms [32]. For example, Capdevila and colleagues [33] have shown that recombinant CYP2C11, CYP2C23, and CYP2C24, in that order, have the highest to lowest epoxygenase activity. Similarly, recombinant rat CYP2J3 and CYP2J4 are active for EETs production [34, 35]. EETs are metabolized by sEH into DHETs [36]. Thus, the action of sEH is to limit the physiological effects of EETs because EETs are generally more biologically active than DHETs (Figure 2). For example, 11,12-DHET, a sEH product, has no vasodilatory action, whereas 11,12-EET produces dilation of renal microvessels [37, 38]. However, in some vascular beds, including canine coronary [39], human coronary arterioles [40], and murine mesenteric arteries [41], both EETs and DHETs cause vasodilation. The rapid development of selective inhibitors of sEH in the past decade is noteworthy; sEH inhibitors have been consistently demonstrated to exhibit protective effects in cardiovascular [42, 43] and renal [44, 45] diseases.

In the brain, EETs production is found in numerous sites, including neurons, astrocytes, and cerebral blood vessels. These lipid effector molecules are important in coupling neuronal activity and astrocytes to induce dilatory responses in cerebral arterioles [46]. For example, in astrocytes, CYP2C11, the major rat epoxygenase enzyme, can produce EETs in the astrocytes [47]. Previous studies have demonstrated that EETs produced in the astrocytes promote opening of astrocyte K_{Ca} channels [48–50], which can generate sufficient K^+ to open inward-rectifier K^+ channels in vascular smooth muscle and thereby elicit hyperpolarization and dilation in cerebral arterioles. Moreover, EETs can be released from astrocytes, and they act in a paracrine manner to open Ca^{2+}-sensitive K^+ (K_{Ca}) channels, hyperpolarize vascular smooth muscle cells, and cause vasodilation in cerebral arterioles [46, 51]. To determine the importance of EETs in cerebral circulation, one interesting study [52] showed that superfusion with MS-PPOH, a selective epoxygenase inhibitor, and miconazole, a reversible CYP inhibitor, significantly attenuated cerebral blood flow during whisker stimulation. However, neither MS-PPOH nor miconazole affected baseline cerebral blood flow. Similarly, Peng *et al.* [53] have found that MS-PPOH attenuated cerebral blood flow during forepaw stimulation. Taken together, these findings establish a role of EETs in regulating cerebral blood flow to neural activation.

3. Role of COX- and CYP-eicosanoids in stroke

3.1. Role of COX-eicosanoids in ischemic stroke

Numerous studies have demonstrated that inflammation is a major contributor to the pathological damage that occurs after ischemic stroke. Following cerebral ischemia, inflammatory cells such as neutrophils infiltrate into the ischemic brain, which triggers the release of chemokines and pro-inflammatory cytokines [7]. It is well recognized that the inflammatory response following cerebral ischemia causes the upregulation of COX-2 expression and increased synthesis of inflammatory PGs [7]. Thus, major research in this area has focused on whether COX-2 blockade or KO is protective against ischemic brain damage. Nagawa *et al.* [54] showed that the expression of COX-2 is upregulated in the injured hemisphere after cerebral ischemia and that administration of NS-398, a selective COX-2 inhibitor, attenuated the elevation of PGE_2 synthesis in the post-ischemic brain and reduced infarct volume by 29% in rats. Another study [55] showed that treatment with NS-398 after ischemic stroke reduces infarct volume and improves neurologic deficits in mice. Notably, COX-2 KO has beneficial effects in the brain injury produced by MCAO, and the protection by COX-2 KO is attributed to the reduction of glutamate neurotoxicity [56]. These findings suggest that COX-2 inhibitors or COX-2 KO exert beneficial effects in ischemic stroke.

3.2. Role of selective COX-2 inhibitors (coxibs) in adverse stroke events

It is estimated that doses of more than 30 billion nonsteroidal anti-inflammatory drugs (NSAIDs) are purchased over-the-counter annually in the U.S. [57]. However, use of conventional NSAIDs is often associated with significant gastrointestinal complications such as ulcers and bleeding. Based on strong data that COX-2 is the major enzyme for the production of inflammatory PGE_2 and that COX-1 is a key enzyme in the production of cytoprotective PGs in the stomach [58], the Food and Drug Administration (FDA) has

approved the use of three coxibs (selective COX-2 inhibitors), rofecoxib, celecoxib, and valdecoxib. Coxibs were developed to reduce the side effects of NSAIDs [58]. While substantial evidence from clinical trials (Adenoma Prevention with Celecoxib (APC), Adenomatous Polyp Prevention on Vioxx (APPROVe), and Prevention of Colorectal Sporadic Adenomatous Polyps (PreSAP) trial) demonstrate that coxibs reduce or prevent the incidence of colorectal cancer [58], long-term use of rofecoxib is also associated with an increased risk of side effects, including increasing incidence of stroke [59]. Because of these unfavorable side effects, rofecoxib and valdecoxib were withdrawn from the market and NIH halted clinical trials of the use of coxibs in 2004. Currently, celecoxib (Celebrex), which is less potent than rofecoxib, is the only coxib on the market, but the black-box warning states that celecoxib may cause risk of stroke.

Numerous reports [60–70] indicate that coxib-induced stroke events are a major obstacle to prolonged use of these drugs. The prevailing theory to explain the adverse cardiovascular effects of coxibs is that they reduce the production of PGI_2, a potent inhibitor of platelet aggregation (Figure 1), but do not affect the production of TXA_2, a potent platelet-aggregating agent (Figure 1) [71]. Thus, stroke complications caused by coxibs might be a consequence of a shifting balance between the levels of PGI_2 and TXA_2 [72]. Although this theory is attractive, it cannot fully explain why other nonselective COX inhibitors, including diclofenac, ibuprofen, naproxen, and indomethacin, also significantly increase the risk of side effects [73]. Hence, more complex mechanisms may be responsible for the coxib-induced stroke events.

Recent work by Zhang *et al.* [58] has provided the first evidence that anti-tumor therapy with rofecoxib induces circulating 20-HETE levels. One could predict that the increased 20-HETE levels are caused by elevation of the substrate, AA, after blocking COX-2. However, rofecoxib did not affect the levels of EETs/DHETs, 5-HETE, 8-HETE, 11-HETE, or 15-HETE. These results are consistent with the previous finding [73] that rofecoxib administration markedly increased 20-HETE levels, but it did not affect circulating levels of EETs, HETEs, LXA_4, and PGD_2. Intriguingly, Zhang *et al.* [58] further showed that 20-HETE blockade by HET0016 prevented stroke event, hemorrhagic transformation, induced by rofecoxib. Since 20-HETE significantly increases platelet aggregation [73] and has detrimental effects on cerebral circulation, it is possible that accumulation of 20-HETE levels after prolonged treatment with rofecoxib contributes to coxib-induced stroke events. Therefore, these findings support the concept that 20-HETE blockade could be a promising approach to prevents coxib-induced cerebral ischemic damage and stroke events.

3.3. Role of EP receptors in ischemic stroke

As mentioned previously, inflammation is a major contributor to the pathological damage occurring following ischemic stroke. PGs are important inflammatory mediators produced in the brain during cerebral ischemia. The deleterious effects of COX-2 activation following stroke have been confirmed in ischemic stroke model. In addition, substantial evidence has demonstrated that inhibition or deletion of COX-2 is cerebroprotective against ischemic stroke [74]. Unfortunately, long-term clinical trials have reported adverse effects following use of coxibs, including an increased risk of stroke and myocardial infarction [59].

Therefore, extensive research has focused on whether downstream EP receptors exert cerebroprotective effects against ischemic stroke.

It is well known that the action of PGE_2 in the brain is mediated by specific G-protein-coupled EP1 to EP4 receptors (Figure 1), which are involved in various physiologic and pathophysiologic conditions [11]. In peripheral blood vessels, the activation of EP1 receptor causes vasoconstriction, leading to the hypothesis that EP1 exerts deleterious effects in ischemic stroke. To test this hypothesis, Saleem *et al.* [75] used MCAO model to study the role of EP1 KO in cerebral blood flow and neuronal cell death during ischemic stroke. They showed that after ischemic stroke cerebral blood flow in EP1 KO mice was significantly elevated relative to wild-type mice. This suggests that EP1 is important in regulating cerebral vascular tone during cerebral ischemia. Moreover, neuronal cultures derived from EP1 KO were more resistant to *tert*-butyl hydroperoxide-induced injury than were neurons from wild-type mice. Another study [76] demonstrated that pretreatment with ONO-8713, an EP1 receptor antagonist, showed notable benefit by reducing infarct size as compared with vehicle group. Additionally, a previous study [77] showed that EP1 receptor blockade improved the survival of hippocampal slices by preventing the attenuation in AKT activity induced by oxygen glucose deprivation (OGD). These findings support the notion that EP1 receptor has a significant function in regulating cerebral blood flow and neuronal cell death, and that EP1 blockade may improve cerebral blood flow and prevent neuronal survival in ischemic stroke.

It is well established that the activation of EP2 receptor triggers cAMP production. Also, EP2 is expressed in neurons of the cerebral cortex, striatum, and hippocampus as well as in the cerebral vasculature [11]. Several studies have demonstrated that activation of EP2 has notable benefit in paradigms on NMDA toxicity and oxygen glucose deprivation [11, 60]. In peripheral blood vessels, the activation of EP2 receptor causes vasodilation, leading to the hypothesis that EP2 exerts cerebroprotective effects against ischemic stroke. To test this, an interesting study by McCullough *et al.* [78] showed that using MCAO model, EP2 KO led to increased cerebral infarction in the cerebral cortex and subcortical structures. This study also showed that EP2 KO did not alter cerebral blood flow after ischemic stroke, which suggests that EP2 KO does not alter the severity of ischemic insult. Another interesting study [79] showed that EP2 KO caused significant increase in stroke volume. Moreover, Li *et al.* [80] demonstrate that treatment with misoprostol, which activates both EP2 and EP4 receptors, caused significant reduce of infarct volume at 24 and 72 h after MCAO. Taken together, these results support the notion that activation of EP2 receptor is cerebroprotective against ischemic stroke.

It is well established that the activation of EP3 receptor decreases cAMP production and that EP3 is expressed mainly in the hypothalamus [11]. To investigate the role of EP3 receptor in ischemic stroke, Saleem *et al.* [81] used MCAO in EP3 KO and wild-type mice. Although EP3 KO led to a decrease in infarct volume at 48 h after ischemic stroke, it did not affect infarct volume at 96 h after ischemic stroke. In contrast, Ahmad *et al.* [82] showed that treatment with ONO-AE-248, a selective EP3 agonist, dose-dependently increased infarct size in MCAO model, suggesting that EP3 activation exerts injurious effects in ischemic

stroke. Given these conflicting findings, the question of whether activation of EP3 has cerebroprotective or injurious effects in ischemic stroke remain unsolved.

It is well established that EP4 activation increases cAMP production. Moreover, EP4 activation causes vasodilation in vascular beds [83], which is associated with activation of endothelial NOS and NO-mediated relaxation of smooth muscle [11, 84]. These findings have led to the hypothesis that EP4 activation is cerebroprotective against ischemic stroke. To test this, an interesting study by Liang *et al.* [85] showed that both endothelial- and neuronal-specific KO of EP4 exacerbated stroke injury and decreased cerebral reperfusion in MCAO model. Moreover, treatment with AE1-329, an EP4 agonist, reduced infarct volume and improved behavioral performance 7 days after ischemic stroke. Also, the activation of EP4 by AE1-329 is associated with elevation of the expression of eNOS in cerebral microvessels. Similarly, treatment with L-902688, an EP4 agonist, caused reduction of infarct volume at 48 h post stroke [86]. Interestingly, a previous study [87] demonstrated that conditional KO of EP4 in macrophages and microglia increased lipid peroxidation and pro-inflammatory gene expression in brain, whereas the treatment with an EP4 agonist decreased LPS-induced pro-inflammatory gene expression in hippocampus and in isolated adult microglia, suggesting the beneficial effect of EP4 receptor signaling in suppressing brain inflammation. Taken together, these novel findings provide the first evidence that EP4 receptor exerts neuronal and vascular protection in ischemic stroke.

3.4. Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in ischemic stroke and subarachnoid hemorrhage

Substantial evidence demonstrates that the impairment in microcirculation significantly contributes to cerebral ischemic damage, and that microcirculation in the peri-infarct region is an important target for the treatment of cerebral ischemia [88]. It is widely accepted that 20-HETE has detrimental effects [89–92] on cerebral circulation, including that it causes vasoconstriction of cerebral arteries; it contributes to the development of cerebral vasospasm; and it increases neuronal excitotoxicity *in vivo* (Figure 2). Extensive research has focused on whether 20-HETE blockade has any beneficial effects against ischemic stroke. Tanaka *et al*. [93], in an early study, made the noteworthy finding that an elevation of 20-HETE levels occurred in the plasma and brain after cerebral ischemia in rats. It is possible that such increased levels were associated with the impairment of cerebral autoregulation after stroke. Importantly, other studies have demonstrated that 20-HETE blockade by TS-011 reduced infarct volume and improved the neurological outcome in rat and monkey stroke models [88, 93, 94].

To further investigate the effects of 20-HETE blockade on cerebral microcirculatory regulation, Marumo *et al.* [90] used an *in-vivo* two-photon imaging system to determine cerebral blood flow after ischemic stroke. They showed that in a vehicle-treated control group, blood flow velocity was significantly decreased at 1 and 2 h after reperfusion, at 4 h after reperfusion, blood flow velocity returned to preocclusion values, but fell again at 7 h after reperfusion. Interestingly, in the TS-011-treated group, blood flow velocities at all time-points after reperfusion were almost identical to the preocclusion value. Although these findings support the notion that 20-HETE blockade improves defects in the regulation of

peri-infarct microcirculation, other studies have shown that 20-HETE blockade does not affect cerebral blood flow. For example, Renic *et al.* [89] showed that although 20-HETE blockade by TS-011 reduced cortical infarct volume, this treatment did not affect CBF during or up to 30 mins after the ischemic period. Similarly, a previous study [95] reported that 20-HETE blockade by HET0016 did not affect pial arteriolar dilation during transient MCAO, suggestion that 20-HETE blockade does not affect cerebral microcirculation after ischemic stroke. Thus, the contribution of 20-HETE in regulating cerebral blood flow after ischemic stroke remains unresolved, and this subject requires further investigation.

It has been postulated that the beneficial effects of 20-HETE blockade in cerebral ischemia are the result of a reduction in oxidative stress and inhibition of apoptosis because in ischemic renal injury 20-HETE increases the generation of superoxide, resulting in apoptosis and cell death of renal cells [96], and 20-HETE blockade attenuated apoptosis after myocardial ischemia reperfusion injury [97]. To support this hypothesis, Renic *et al.* [98], using organotypic hippocampal slices after oxygen-glucose deprivation, have demonstrated that 20-HETE contributes to neuronal death through the generation of reactive oxygen species and activation of caspase-3.

As noted earlier, hemorrhagic stroke accounts for 15%-20% of all stroke incidents. Cerebral vasospasm is an important pathological event that occurs in the early phase of hemorrhagic stroke. Interestingly, substantial evidence suggests that 20-HETE constricts cerebral arteries, regulates new blood vessel growth, augments vascular remodeling, and contributes to the development of acute and delayed cerebral vasospasm [99–101]. Thus, many research has focused on the role of 20-HETE in SAH. By injecting arterial blood into the cisterna magna to mimic SAH, Kehl *et al.* [3] showed that 20-HETE blockade by HET0016 reduced the initial fall in cerebral blood flow by 40% after SAH. Moreover, they further showed that the concentration of 20-HETE in the cerebrospinal fluid rose from 12 to 199 ng/ml after SAH. These results demonstrate that 20-HETE plays an important role in SAH. Another study [102] has demonstrated that the development of delayed vasospasm after SAH in dogs was associated with the elevation of 20-HETE levels in cerebrospinal fluid and that 20-HETE blockade by TS-011 reversed delayed vasospasm in this model. Interestingly, a recent study by Crago *et al.* [99] determined whether 20-HETE levels in the cerebrospinal fluid are associated with delayed cerebral ischemia in patients with SAH. This report [99] showed that 20-HETE levels were observed in 31% of patients with SAH; also, the levels of 20-HETE were associated with delayed cerebral ischemia. These findings provide important clinical evidence that inhibiting 20-HETE synthesis could be a useful therapeutic intervention in patients with SAH.

3.5. Role of soluble epoxide hydrolase (sEH) in ischemic stroke and subarachnoid hemorrhage

Substantial evidence shows that EETs are important mediators that cause vasodilation in cerebral arterioles, regulate cerebral blood flow and neurovascular coupling, promote angiogenesis, and have protective effects against ischemia [2, 46, 51, 103]. However, chronic treatment with exogenous EETs in *in-vivo* experiments is impractical because EETs are rapidly degraded by sEH (Figure 2) [32]. Therefore, researchers have used sEH blockade

or KO to increase EETs systemically and to investigate their effects in ischemic stroke. To examine the role of EETs in hypertension-induced ischemic stroke, Dorrance *et al.* [104] demonstrated that treatment with AUDA, a selective sEH blocker, significantly reduced infarct size in stroke-prone spontaneously hypertensive rats (SHRSP) with cerebral ischemia, but it did not affect the blood pressure of SHRSP. Thus, sEH blockade is protective against cerebral ischemia in a blood-pressure-independent manner in SHRSP. Moreover, Simpkins *et al.* [105] reported that using MCAO model, sEH blockade reduced hemispheric infarct size, reduced wall-to-lumen ratio and collagen deposition, increased cerebral microvessel density, and reduced the expression of apoptotic factors in SHPSR. These findings support the notion that sEH blockade against cerebral ischemia via vascular and neural protection in SHRSP rats. Notably, several other interesting studies have demonstrated that sEH blockade is cerebroprotective in mouse model of ischemic stroke [106], rat model of ischemic stroke [107], type-1 diabetic stroke model [108], and type-2 diabetic stroke model [109].

To further investigate the role of sEH in cerebral circulation during ischemic stroke, Zhang *et al.* [110] determined the impact of sEH KO with 2-hour MCAO, showing that sEH KO significantly reduced infarct size and improved regional cerebral blood flow rates after cerebral ischemia. Importantly, they further showed that 14,15-EET infusion reduced infarct size. These findings demonstrate that sEH KO is protective against ischemic stroke by a cerebrovascular mechanism. Furthermore, a recent study [111] has determined the role of sEH in the endothelium using transgenic mice with endothelial-specific expression of human sEH (Tie2-hsEH) against cerebral ischemia. This study showed that following 1 μM acetylcholine administration, cerebral blood flow was significantly reduced in Tie2-hsEH mice as compared to male wild-type mice [111]. Although no difference in infarct size was observed in male Tie2-hsEH and wild-type mice, female Tie2-hsEH mice exhibited larger infarct volumes than male in the striatum after ischemic stroke. These findings provide solid evidence that endothelial sEH is a key player in regulating cerebrovascular endothelial function as well as an important contributor to the sexually dimorphic response to cerebral ischemia.

Cerebral edema, which occurs as a result of a disruption of the blood-brain barrier, is associated with the activation of vascular inflammatory cascade, and it is a common and serious complication of hemorrhagic stroke [112]. Since EETs decrease VCAM-1 expression in cerebral microvessels and regulate dilatory responses in cerebral arterioles, recent studies [112, 113] have focused on the role of sEH in SAH. Using endovascular puncture to mimic SAH, Siler *et al.* [112] recently reported that wild-type mice exhibited tissue edema within 6 hours and the peaked at 24 hours, which was determined by T2-weighted MRI images, after SAH. They also demonstrated that sEH KO significantly reduced edema, reduced overall VCAM-1 uptake, and improved functional outcome after SAH as compared with wild-type mice. These findings demonstrate that sEH KO reduces vascular inflammation and edema and improves outcome after SAH. To further define the role of EETs in patients with SAH, Siler *et al.* [113] recently documented that 14,15-EET levels were significantly elevated in the cerebrospinal fluid of patients who had suffered SAH as compared to control subjects. This study [113] also showed that sEH KO caused

higher levels of 14,15-EET relative to control mice in the whole brain and had the cerebroprotective effects from the delayed decrease in microvascular cortical perfusion after SAH. These findings demonstrate that increasing EETs levels may act as an endogenous protective response against delayed cerebral ischemia after SAH. Together, these findings provide important evidence that either inhibiting sEH or increasing EETs levels could serve as a comprehensive approach to preventing the development of delayed cerebral ischemia in SAH.

1. Conclusion

Substantial evidence has demonstrated that cerebral vascular function and dysfunction are the key factors in the onset and progression of ischemic and hemorrhagic stroke. COX and CYP-derived eicosanoids are important lipid mediators that mediate cerebral vascular tone, cerebral blood flow, and autoregulation of cerebral circulation. These lipid mediators have important functions in ischemia-induced damage. In this review, we have outlined the role of COX- and CYP-derived eicosanoids in cerebral vascular function as well as their role in ischemic stroke and SAH.

Two COXs isoforms, COX-1 and COX-2, are expressed in the brain. COX-1 is constitutively expressed, and COX-2 is highly inducible after ischemic stroke. It appears that COX-1 is involved in regulating resting cerebral blood flow, whereas COX-2 is involved in the increase of cortex blood flow that accompanies neural activity. Although animal studies have shown that inhibition or deletion of COX-2 is cerebroprotective against ischemic stroke, many clinical data demonstrate that long-term treatment with coxibs is associated with increased risk of side effects, including stroke and myocardial infarction. For this reason, research has begun to be focused on delineating the role of downstream EP receptors in ischemic stroke. Accumulating evidence has demonstrated that activation of EP2 and EP4 receptors exerts cerebroprotection in ischemic stroke. Therefore, the development of novel pharmacological agents targeting EP2 and EP4 receptors could be an important area for clinical studies in patients with ischemic stroke.

20-HETE is the major CYP product in the cerebral circulation. It is well known that CYP4A and CYP4F isoforms are the major enzymes for 20-HETE synthesis and that these enzymes are expressed in cerebral microvessels. Substantial evidence demonstrates that 20-HETE, a highly potent vasoconstrictor in cerebral arteries, depolarizes vascular smooth muscle cells by inhibiting K^+ channel activity and is active in the autoregulation of cerebral blood flow. It appears that 20-HETE has detrimental effects in the brain. Also, growing evidence demonstrates that 20-HETE blockade improves defects in the autoregulation of peri-infarct microcirculation and is cerebroprotective against ischemic stroke and SAH. Although some evidence suggests that the action of 20-HETE is mediated through a 20-HETE receptor, that receptor has yet to be identified. Additional studies are needed to identify 20-HETE receptor, which will facilitate future studies in the role of 20-HETE in stroke research.

EETs, which are CYP2C and CYP2J-derived eicosanoids, are important lipid mediators in cerebral circulation. Substantial evidence demonstrates that EETs cause vasodilation in cerebral arterioles, regulate cerebral blood flow and neurovascular coupling, and have

protective effects against ischemia. Since EETs are rapidly degraded by sEH, much research has focused on the role of sEH blockade and KO to increase systematic EETs levels. Evidence is accumulating that endothelial sEH is a key player in regulating cerebrovascular endothelial function and contributes to the sexually dimorphic response to cerebral ischemia. sEH blockade provides cerebroprotection in SAH. It is important to note that a recent study revealed that EET analog has beneficial effects in hypertension and renal protection [114]. Thus, the development of novel EET analogs for *in-vivo* studies will be an interesting approach in investigating the role of EETs in ischemic stroke and SAH.

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Highlights

- Long-term use of COX-2 inhibitors (coxibs) increase risk of stroke, and 20-HETE blockade is a possible strategy to prevent coxib-induced stroke events.
- Activation of EP2 and EP4 receptors exerts cerebroprotection against ischemic stroke.
- 20-HETE has detrimental effects in the brain, and that its blockade exerts cerebroprotection against ischemic stroke and subarachnoid hemorrhage (SAH).
- sEH blockade is cerebroprotective against ischemic stroke and SAH.

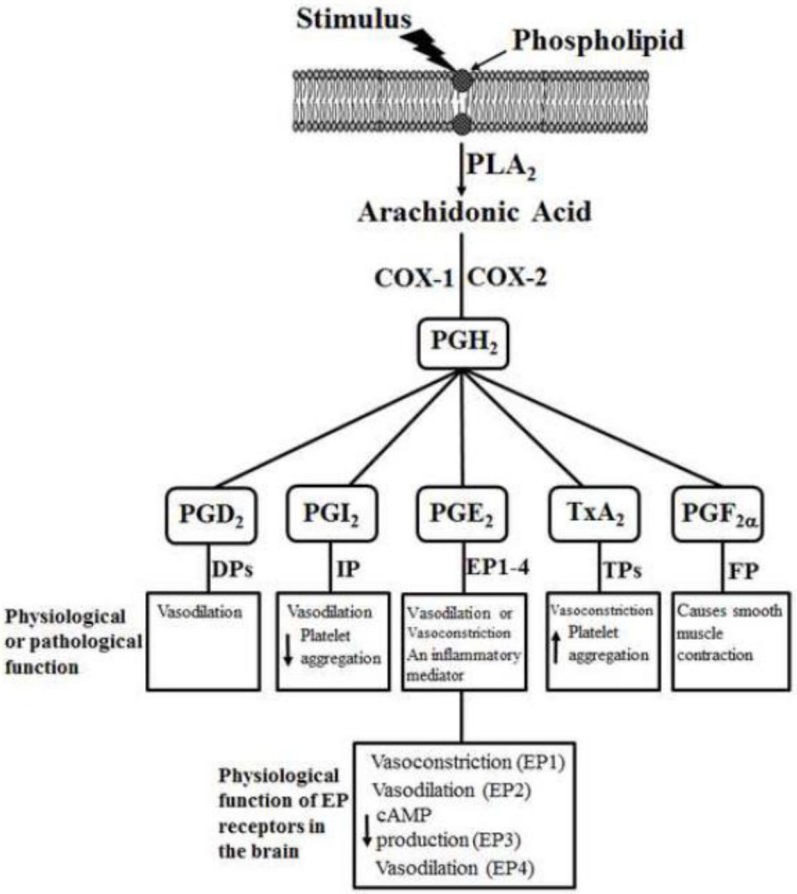

Figure 1.

Cyclooxygenase (COX)-derived eicosanoids from the arachidonic acid (AA) cascade. After trigging by inflammatory conditions such as the presence of cytokines and growth factors, AA-containing phospholipids are hydrolyzed by phospholipase A_2 (PLA_2) resulting in the release of free AA. AA can be further metabolized by COX-1 or COX-2 to PGH_2. The generation of PGD_2, PGI_2, PGE_2, TxA_2, and $PGF_{2\alpha}$ is mediated through tissue-specific isomerases and synthases. The action of these PGs and TXA_2 is mediated via DPs, EPs (EP_1 to EP_4), IP, FP, and TPs receptors. The physiological or pathological function of these eicosanoids is also summarized in Figure 1. We also indicate the physiological function of EP receptors in the brain in Figure 1.

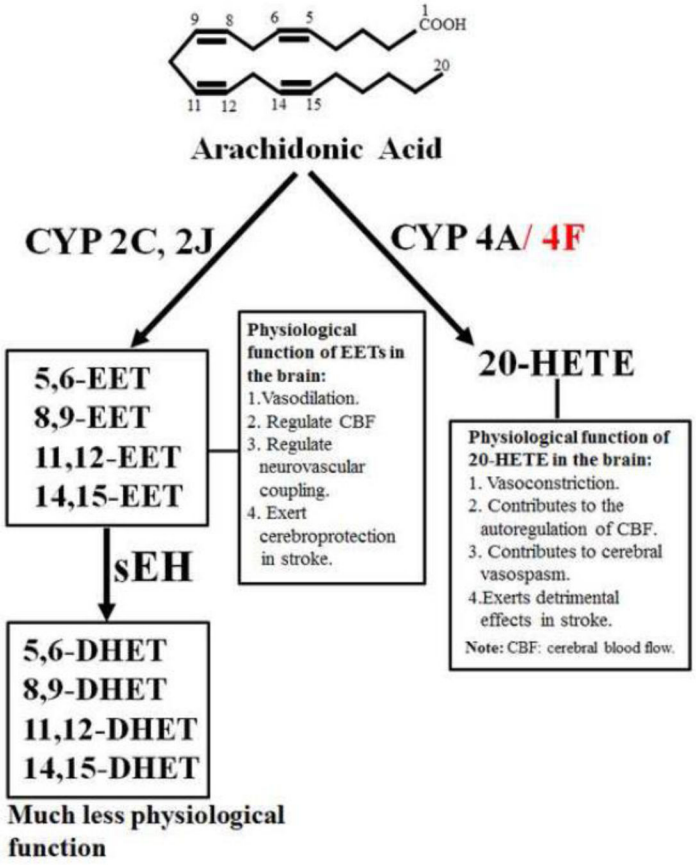

Figure 2.

The metabolic pathway of arachidonic acid by cytochrome P450 (CYP) enzymes. 20-HETE, 20-hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; DHET, dihydroxyeicosatrienoic acid; sEH: soluble epoxide hydrolase. The physiological function of 20-HETE and EETs in the brain is also summarized in Figure 2.