Humble beginnings with big goals: Small molecule soluble epoxide hydrolase inhibitors for treating CNS disorders

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Abstract

Soluble epoxide hydrolase (sEH) degrades epoxides of fatty acids including epoxyeicosatrienoic acid isomers (EETs), which are produced as metabolites of the cytochrome P450 branch of the arachidonic acid pathway. EET inhibition is shown to be therapeutic in several cardiovascular and renal disorders, as well as in peripheral analgesia, via the increased availability of anti-inflammatory EETs. The success of sEH inhibitors in peripheral systems suggests their potential in targeting inflammation in the central nervous system (CNS) disorders. Here, we describe the current roles of sEH in the pathology and treatment of CNS disorders such as stroke, traumatic brain injury, Parkinson’s disease, epilepsy, cognitive impairment, dementia and depression. In view of the robust anti-inflammatory effects of stem cells, we also outlined the potency of stem cell treatment and sEH inhibitors as a combination therapy for these CNS disorders. This review highlights the gaps in current knowledge about the pathologic and therapeutic roles of sEH in CNS disorders, which should guide future basic science research towards translational and clinical applications of sEH inhibitors for treatment of neurological diseases.

Keywords:
Pharmacology
Stroke
TBI
Inflammation
Preclinical studies
Clinical trials
Druggability

1. Introduction

Three inflammatory pathways that produce eicosanoids derived from arachidonic acid (ARA) have been implicated in a variety of inflammation-plagued disorders such as stroke, hypertension, and renal disease. The first pathway entails cyclooxygenase (COX) enzymes that are responsible for the conversion of ARA to prostaglandins and thromboxane (a potent vasoconstrictor). Both aspirin, prescribed for at-risk stroke patients, and non-steroidal anti-inflammatory drugs are COX inhibitors (Vane, 1971). The second pathway consists of lipoxygenase (LOX) enzymes that are required to produce pro-inflammatory leukotrienes from ARA, and LOX inhibitors have been used therapeutically in seasonal allergies and asthma (Cegielska-Perun et al., 2016; Ribeiro et al., 2006). The third pathway is mediated by cytochrome P450 enzymes (CYP) and results in two types of products: hydroxyeicosatrienoic acids (HETEs) and epoxyeicosatrienoic acid isomers (EETs). EETs are produced by the metabolic activity of CYP enzymes which among other metabolites produce epoxides from double bonds (Capdevila et al., 2000). These compounds have been shown to exert potent, protective vasodilatory and anti-inflammatory effects on the cardiovascular and renal systems (Spector et al., 2004). Moreover, EETs also suppress hyperthermia, pathological fibrosis, the generation of reactive oxygen species, apoptosis, pain, and platelet aggregation (Zhang et al., 2007; Xu et al., 2013; Morisseau and Hammock, 2013; Yang et al., 2015a; Harris and Hammock, 2013; Yang et al., 2015b). Although the beneficial effects of EETs on the cardiovascular and renal systems have taken precedence over their roles in other systems, they assume an important role in central nervous system (CNS) signaling as well (Ilfif et al., 2010). While many of the effects of EETs on the CNS parallel their effects on peripheral systems, recent research reveals that...
EETs have distinct functions in the brain, which may have anti-inflammatory effects. Some of these functions relevant to regulation of inflammation include the modulation of angiogenesis, regulation of cerebral blood flow (CBF), and mediation of neuroendocrine signaling (Illif et al., 2010).

Soluble epoxide hydrolase (sEH), first assayed (Mumbey and Hammock, 1979a, b) and characterized (Gill and Hammock, 1979; Grant et al., 1994) almost 40 years ago, is responsible for the degradation and conversion of EETs into dihydroxyeicosatrienoic acids (DHETs), which have diminished and different biological effects compared to EETs (Spector et al., 2004; Fang et al., 2004). Furthermore, sEH inhibitors (sEHIs) harbor therapeutic potential by increasing the availability of EETs and other epoxy fatty acids (EpFAs) (Shen, 2010; Yu et al., 2000). In the last two decades, sEHs have enjoyed a rapid ascent into clinical research (Table 1) since their groundbreaking capacity to produce anti-hypertensive effects was discovered (Imig and Hammock, 2009; Imig et al., 2002). The aforementioned anti-hypertensive effects were then attributed to the ability of sEHIs to inhibit the actions of hormone angiotensin-II (Jung et al., 2005). This finding not only bolstered previous research on the potential treatment of hypertension with sEHIs, but also elucidated the relationship between sEHs and the renal system. With the advent of more potent and bioavailable inhibitors, sEHs entered clinical studies to evaluate their safety and efficacy for health problems such as hypertension and type 2 diabetes mellitus. In a phase Ia clinical trial, the sEH AR9281 demonstrated decent tolerability, rapid absorption, no severe side effects, and a 90% inhibition rate of sEH activity in the blood (Chen et al., 2012). Additionally, combining sEHIs with the vasoconstrictor

Table 1

<table>
<thead>
<tr>
<th>Study</th>
<th>Discovery</th>
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</thead>
<tbody>
<tr>
<td>Gill et al., 1974</td>
<td>Incubation of the juvenile 1-(4'-ethylenoxy)-6,7-epoxy-3,7-dimethyl-2-oxo-1,1-dimethyl-2-cyclopentene with rat liver microsomes forms dio1 metabolites and is the first description of sEH in mammals</td>
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<tr>
<td>Hammock et al., 1976</td>
<td>Various experiments reveal sEH's biochemical characteristics such as an isoelectric point of 4.9, a molecular weight of 150,000, and increased activity in kidney and liver soluble fractions relative to microsomal fractions</td>
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<tr>
<td>Mumbey and Hammock, 1979a,b</td>
<td>In mouse livers, epoxide hydrolase-mediated hydration rates change depending on the quantity of alkyl substitutions on the epoxide, exhibiting sEH substrate specificity for the first time</td>
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<tr>
<td>Gill and Hammock, 1979</td>
<td>sEHs hydrate both cis- and trans- epoxyethyl steares in mouse livers, especially in the cytosolic fraction; the first description of epoxy fatty acids as substrates</td>
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<tr>
<td>Gill and Hammock, 1980</td>
<td>First comprehensive description of sEH, revealing that epoxide hydrolase activity is present in various organs like the spleen, lung, colon, etc., but is highest in the liver and kidney; is higher in male mice compared to female mice, increases as mice age, does not require a cofactor, and is inhibited by inorganic ions</td>
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<tr>
<td>Ota and Hammock, 1980</td>
<td>sEH (cytosolic) activity, unlike microsomal enzymes, was overlooked by other researchers because assays with high pH were utilized, which minimizes sEH activity; sEH activity is lowest in rats compared to mice and guinea pigs, but rats are frequently used for epoxide hydrolase distribution investigations; and studies with streyne oxide led to the misconception that epoxide hydrolase activity requires membrane binding</td>
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<tr>
<td>Haegawa and Hammock, 1982</td>
<td>A spectrophotometric assay using substrate trans-stilbene oxide describes the measurement of mammalian cytosolic epoxide hydrolase activity</td>
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<tr>
<td>Grant et al., 1993</td>
<td>Structural analysis of the isolated sEH coding sequence reveals the first characterization and transient expression of the cloned cDNA in cell culture</td>
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<td>Grant et al., 1994</td>
<td>Chromosomal location of murine sEH gene is determined at band D of chromosome 14, homologous to human chromosomes 8, 13, and 14</td>
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<tr>
<td>Pinot et al., 1995</td>
<td>sEH activity in mice is higher in males than in females. sEH is under regulation by peroxisome proliferators and hormones such as testosterone.</td>
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<td>Draper and Hammock, 1999a,b</td>
<td>Zinc and other metals can act as inhibitors of sEH, suggesting a mechanism of enzyme down-regulation during inflammation</td>
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<tr>
<td>Draper and Hammock, 1999a,b</td>
<td>sEH activity present in rat inflammatory cells is indistinguishable from rat liver cytosolic sEH</td>
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<tr>
<td>Yu et al., 2000</td>
<td>EETs are involved in renal vasculature activity, thus the use of an sEH inhibitor to decrease EET hydrolysis is a novel therapy for regulating blood pressure</td>
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<tr>
<td>Alkayed et al., 2002</td>
<td>Upregulation of EETs post ischemic stroke is an innate mechanism to protect the brain against future damage</td>
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<tr>
<td>Morisseau et al., 2002</td>
<td>Lipophilicity controls the water solubility of sEH inhibitors, which limits their potency as regulators of blood pressure and inflammation</td>
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<tr>
<td>Borrance et al., 2005</td>
<td>sEH knockout mice, with EPHX2 gene deletion, demonstrate that sEH gene deletion protects against ischemic stroke via a vascular mechanism that reduces EET hydration</td>
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<td>Liu et al., 2005</td>
<td>sEH inhibition post OGD increases EET and VEGF levels, indicating that astrocytes may help control sEH-induced neuroprotection</td>
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<td>Schmelzer et al., 2005</td>
<td>sEH inhibitors reduce inflammation by reducing levels of proinflammatory cytokines and nitric oxide metabolites</td>
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<td>Zhang et al., 2007</td>
<td>sEH AUDA-BE reduces infarct size (post ischemic stroke) and is neuroprotective by non-vascular mechanisms</td>
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<tr>
<td>Iliff et al., 2009</td>
<td>EETs aid in neurogenic vasodilatation and are produced by perivascular nerves</td>
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<tr>
<td>Zhang et al., 2009</td>
<td>sEH in part explains sex-linked differences in blood flow and brain damage post ischemic stroke</td>
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<td>Sarkar et al., 2011</td>
<td>Epoxygenase activity is impaired in Alzheimer's disease</td>
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<tr>
<td>Vanella et al., 2011</td>
<td>sEH inhibition, using sRNAs, decreases mesenchymal stem cell-derived adipocyte stem cell differentiation</td>
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<tr>
<td>Fremel et al., 2012</td>
<td>Long-term sEH inhibition is detrimental to hematopoietic progenitor cell proliferation, mobilization, and vascular repair</td>
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<td>Shaik et al., 2013</td>
<td>sEH KO mice display improved behavioral outcomes in comparison to wild-type mice post TBI</td>
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<tr>
<td>Strauss et al., 2013</td>
<td>sEH KO mice display improved behavioral outcomes in comparison to wild-type mice post TBI</td>
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<tr>
<td>Inonoglu et al., 2013</td>
<td>Mice treated with sEHIs are resistant to GABA-antagonist induced seizures</td>
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<tr>
<td>Hung et al., 2015</td>
<td>sEH knockout AUDA attenuates inflammation and decreases seizure susceptibility in a rat model of temporal lobe epilepsy</td>
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<tr>
<td>Li et al., 2015</td>
<td>EETs enhance hematopoietic stem and progenitor cell engraftment migration in mammals</td>
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<td>Qin et al., 2015</td>
<td>Tyrosine hydroxylase-positive cell death can be reduced by sEH deficiency or administration of 14,15-EET in a PD mouse model</td>
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<tr>
<td>Liu et al., 2016</td>
<td>sEHs 14,15-EET and AUDA cause suppressed astroglisis, enhanced angiogenesis, decreased neural apoptosis, and reduced glial scar formation in vivo</td>
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<tr>
<td>Ren et al., 2016</td>
<td>sEH inhibitor TPUU mitigates depressive symptoms in mice</td>
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<td>Zhang et al., 2017</td>
<td>sEH inhibition post OGD increases EET and VEGF levels, indicating that astrocytes may help control sEH-induced neuroprotection</td>
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<tr>
<td>Hung et al., 2017</td>
<td>sEH KO mice and AUDA-treated mice display improved behavior, decreased brain edema, less brain tissue damage and apoptosis, and less BBB permeability post TBI</td>
</tr>
<tr>
<td>Lakkappa et al., 2018</td>
<td>sEH inhibitor FTUPB provides neuroprotection in a Drosophila model of PD</td>
</tr>
<tr>
<td>Ren et al., 2018</td>
<td>sEH inhibitor TPUU ameliorates reduction of DA, DOPAC, and HVA in a MPTP-induced mouse model of neurotoxicity</td>
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urotensin II resulted in a larger increase in blood vessel flux in healthy patients and patients with heart failure, suggesting sEHIs’ potential to treat heart failure (Tran et al., 2012). GSK2256294, another sEH, was evaluated in both healthy males and obese smokers in a phase I clinical trial, and was found to have no serious adverse effects, with contact dermatitis and headaches as the most common side effects (Lazaar et al., 2016). The same sEH was also studied in elderly males and females with similar results (Lazaar et al., 2016). Fortified by the extensive research on hypertension and sEH, the putative neuroprotection of sEHs emerged into the field of ischemic stroke. sEH is abundantly expressed in the cerebral cortex, striatum, hypothalamus, and brain stem, predominantly in neuronal cell bodies (Zhang et al., 2007; Sellers et al., 2005) but also in astrocytes and oligodendrocytes (Harris and Hammock, 2013). Coinciding with the benefits of sEHs in hypertension and related cardiovascular disease, sEH activity may be present within the endothelium as well (Zhang et al., 2013a). Indeed, the inhibition of sEH attenuated inflammation and was associated with a smaller infarct size after middle cerebral artery occlusion (MCAO) in mice (Zhang et al., 2007). Interestingly, sEH immunoreactivity was confirmed in both non-vascularized and vascularized areas of the brain, suggesting that the beneficial actions of sEHs may similarly comprise of vascular as well as non-vascular pathways. Due to the robust ability of sEHs to suppress the inflammatory response, sEHs have become increasingly relevant in the possible treatment of CNS disorders (Fig. 1). Other mechanisms by which sEHs may have beneficial effects in the treatment of CNS disorders have also been unveiled, and they will be discussed in detail below.

In the present review, we seek to describe the current knowledge surrounding the potential role of sEHIs in treatment for CNS disorders such as stroke, traumatic brain injury (TBI), Parkinson’s disease (PD), and other debilitating neurological conditions. Inflammation is a major mediator of cell death in the diseases mentioned above, and we will explore the anti-inflammatory mechanisms of sEHs that serve as the basis for combating these disorders. In addition, we hope to synthesize research conducted on the intersection of sEH inhibition and stem cell treatment. The treatment of CNS diseases with stem cells has reached clinical trials, but to date, most of the reported findings relate to safety with limited efficacy and may benefit from combination therapy, such as with sEHIs. While the anti-inflammatory effect of sEHs observed in other body systems is a promising therapeutic tool for use in CNS disorders, unique properties of the brain and complex pathological processes of each neurological disorder may hinder their swift integration into clinical practice. In this review, we will provide an outline of possible and current challenges sEHs may face on their way into the clinic. We also hope to highlight the gaps in current scientific and translational knowledge to provide guidance for future clinical studies geared towards advancing stem cell therapy and pharmacological sEHI treatment for neurological disorders.

2. Distribution of sEH

sEH is present in inflammatory cells and throughout multiple organs in mammals, including the liver and kidney (Chiamvimonvat et al., 2007; Draper and Hammock, 1999a). It can be found in the endothelium and smooth muscle of vascular tissues, suggesting the enzyme’s potential connection to hypertension (Chiamvimonvat et al., 2007). Within the cell, sEH is mainly located in the cytosol and peroxisomes, but this cytosolic/peroxisomal distribution changes with tissue type and physiological state (Chiamvimonvat et al., 2007). Interestingly, sEH is also highly prevalent in the cerebral cortex and striatum of the brain and typically resides in neuronal cell bodies, astrocytes, oligodendrocytes, and the smooth muscle of cerebral blood vessels (Zhang et al., 2007; Sura et al., 2008). As stated above, sEH is also found within the endothelium (Zhang et al., 2013a). These localization patterns, particularly sEH’s propensity for endothelial cells and smooth muscle cells of cerebrovasculature, implicate the enzyme’s possible involvement in regulating CBF, brain activity, and neurological disease pathology (Sura et al., 2008). In fact, administering sEHIs decreases sEH activity and mitigates brain damage in stroke and TBI animal models (Zhang et al., 2007; Hung et al., 2017). This implies a neuroprotective effect upon sEH inhibition and advocates the development of potent sEHIs with favorable pharmacokinetic attributes as potential drug treatments for neurological diseases.

The unique distribution of sEH in the human body also reveals sexually dimorphic expression. sEH activity is higher in post-puberty male mice than in females (Gill and Hammock, 1980), suggesting differential regulation by hormones such as testosterone (Pinot et al., 1995) and estradiol (Koerner et al., 2008). Detailed research must be conducted in the future to fully elucidate sex differences in sEH activity and distribution. This concept, along with the distinct impacts that specific neurological disorders have on the sexes, must be acknowledged when developing and evaluating the effects of potential sEHs.

3. Absorption, distribution, metabolism, and excretion (ADME) of sEHIs

Maintaining simplicity of formulation and enhancing ADME properties of sEH inhibitors is crucial to bolstering their efficacy (Fig. 2) (Qiu et al., 2011). Ideal sEHIs maximize bioavailability and delay elimination from the bloodstream as exhibited by longer half-lives, higher maximum drug concentrations in the blood (Cmax), and larger area under the curve (AUC) values than earlier sEHIs such as 12-(3- adamantyl-1-yl-ureido)-dodecanoic acid (AUDA) (Liu et al., 2009). Additionally, lower melting points are beneficial for boosting absorption in the body (Chu and Yalkowsky, 2009) and higher solubility in water improves oral administration and bioavailability (Chiamvimonvat et al., 2007; Kim et al., 2004). Furthermore, decreasing crystalline stability and increasing oil solubility enables sEHIs to experience easier subcutaneous administration and dissolution in organic solvents used to transfer drugs (Chiamvimonvat et al., 2007).

Initial sEHs included dicyclohexylurea (DCU), a potent but highly lipophilic compound with a high melting point. DCU is a byproduct of some industrial and laboratory synthesis procedures. However, DCU’s pharmacological utility is restricted as excessive lipophilicity in a compound generates pharmacokinetic and formulation issues and hinders its specificity (Chiamvimonvat et al., 2007; Iliff and Alkayed, 2009). Adding polar functional groups to early sEHIs like DCU generated a new line of potent, more water soluble sEHIs including AUDA and its butyl ester (AUDA-Be) (Liu et al., 2009). Preclinical pharmacokinetics revealed that AUDA-Be, when injected intraperitoneally in mice, travels systemically to the brain, crosses the blood brain barrier (BBB), and inhibits sEH in the brain (Zhang et al., 2007). sEH activity is inhibited by over 20% for 24 h, confirming AUDA-Be’s potency and bioavailability (Zhang et al., 2007). AUDA-Be appears to exhibit superior bioavailability, but both AUDA and AUDA-Be are capable of crossing the BBB and inhibiting sEHi (Iliff and Alkayed, 2009).

Intriguingly, AUDA-Be and AUDA’s other butyl and ester forms can be transmitted intraperitoneally, subcutaneously, or orally via food, while AUDA can be dosed orally via drinking water (Chiamvimonvat et al., 2007). Adamantane was used in many early sEHIs like AUDA because it could be detected at very low concentrations by liquid chromatography-mass spectrometry (LC-MS) and because it was rapidly metabolized. AUDA compounds encounter swift metabolism via beta oxidation in the body (Chu and Yalkowsky, 2009) and higher solubility in organic solvents used to transfer drugs (Chiamvimonvat et al., 2007). AUDA can be dosed orally via drinking water (Chiamvimonvat et al., 2007). Addition of 1-adamantan-1-yl-3-(5-(2-ethoxyethoxy)ethoxy)pentylurea (AEPU) (Qiu et al., 2011). Like many modern sEHIs, AEPU has a polar functional group located...
Fig. 1. The effects of sEH inhibition on major CNS disorders. sEH inhibition prevents the typical breakdown of EETs and other EpFAs into DHETS or other corresponding 1,2-diols, facilitating the regenerative process of certain disorders. This treatment may be coupled with stem cell therapy in order to improve treatment outcomes.

Fig. 2. Soluble epoxide hydrolase inhibitor (sEHI) structures. The chemical structures of several sEHIs. These compounds inhibit sEH, whose three-dimensional structure is depicted on the right (PDB ID: 1CQZ; MMD8 ID: 11526).
approximately 8 Å away from its central carbonyl group (Chiamvimonvat et al., 2007), allowing higher solubility in water and a lower melting temperature, thereby making AEPU’s formulation more accessible. The inhibitor readily traverses membranes and possesses good bioavailability and effectiveness in vivo (Chiamvimonvat et al., 2007; Qiu et al., 2011). However, it was designed for transient activity and quick metabolic breakdown (Chiamvimonvat et al., 2007). Other new inhibitors, including t-AUCB (trans-4-(4-(3-adamantan-1-yl-ur- eido)-cyclohexyloxy)-benzoic acid) and TPPU (1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea) have succeeded earlier sEHIs with increased water solubility and abated metabolism due to their confined conformational structures (Qiu et al., 2011; Liu et al., 2009). One compound of interest is 1-trifluoromethoxyphenyl-3-(1-propionylpiperidine-4-yl) urea (TPPU), a potent, relatively easily-formulated sEHI with sufficient solubility in water. It is currently the most widely used sEHI in research. TPPU maintains its robust bioavailability in the brain after intraperitoneal injection (Ulu et al., 2016) and oral administration (Ren et al., 2016), demonstrates vast systemic distribution to tissues, effectively crosses the BBB, experiences excellent absorption in the intestines, significantly inhibits sEH, possesses high metabolic stability, and is effectively administered via drinking water but must be formulated in a water miscible organic co-solvent (Ren et al., 2016; Ostermann et al., 2015). Indeed, many sEHIs successfully pass the BBB and each new generation of inhibitors advances the previous generation with improved physical characteristics like increased water solubility. TPPU exhibits much greater stability, bioavailability, half-life duration, and efficacy in clearing inflammation than t-AUCB, appealing properties attributed to the substituted phenyl group attached to TPPU in contrast to the adamantyl group attached to t-AUCB (Liu et al., 2013). However, t-AUCB derivatives are broadly active on the sEH of many mammalian species while TPPU-like compounds are most active on primate and rodent sEHs. These results further emphasize the importance of sEH structure and functional groups, and how these affect inhibitor efficacy and pharmacokinetic properties. It will be imperative to refine sEH structures with this in mind to develop optimized drugs for treating diseases.

While sEHIs like AUDA and TPPU can cross the BBB, their structure has not been specifically optimized for BBB penetration. Lipid solubility is key to crossing the BBB (Banks, 2009), but excessive lipophilicity hinders sEHI formulation, creates pharmacokinetic issues (Chiamvimonvat et al., 2007), and can allow the sEHI to be taken from the bloodstream by other tissues before it reaches the brain (Banks, 2009). Thus, a compromise must be made to improve BBB penetration while maintaining the inhibitor in the bloodstream (Banks, 2009). As low molecular weight and lipid solubility facilitate easier crossing of the BBB, it is possible that increasing hydrogen bonding capabilities or molecular weight by adding additional functional groups in some sEHIs may also prevent complete optimization of BBB crossing (Chiamvimonvat et al., 2007; Banks, 2009). Thus, an ideal balance is necessary to maintain sEHI potency while simultaneously maximizing availability in the brain.

Most central pharmacophores in sEHIs are a urea, carbamate, or amide moiety (Chiamvimonvat et al., 2007). Modifying the secondary pharmacophore by including hydroxyl, carbonyl, sulfonyl, ether, ester, carbamate, and other functional groups enhances the inhibitor’s physical attributes, such as by decreasing melting temperature and increasing binding to polar residues in the sEH catalytic tunnel (Chiamvimonvat et al., 2007). Adding polar groups to inhibitors in the proper position may increase the likelihood of hydrogen bonding with catalytic sites on sEH and improve target selectivity and pharmacokinetic and physical aspects (Chiamvimonvat et al., 2007). For instance, water solubility increases without compromising inhibitor efficacy when polar functional groups are present on a linear alkyl chain at a certain distance from the urea moiety (Kim et al., 2004). Thus, structural modifications of inhibitors can increase solubility in water or oil-based solvents or lower melting points in order to increase bioavailability (Kim et al., 2004, 2007; Morisseau et al., 2002). Augmenting bioavailability following oral administration and minimizing clearance from the bloodstream are current objectives driving the latest structural alterations to sEHIs (Iliff and Alkayed, 2009). Of note, there has been a breakthrough within the past 15 years in promising compounds possessing extensive half-lives and exceptional oral bioavailability (Chiamvimonvat et al., 2007; Iliff and Alkayed, 2009). Pharmaceutical companies can potentially use these compounds as templates for developing highly effective sEHIs to treat a wide variety of diseases, including neurological maladies like TBI and ischemic stroke (Iliff and Alkayed, 2009). In addition to developing sEHIs with slow metabolism and sufficient oral bioavailability, dual COX/sEH inhibitors are of current interest. A COX-2/sEH inhibitor administered to rats shows a synergistic effect in treating lipopolysaccharide induced pain (Hwang et al., 2011). In fact, the compound shows an enhanced effect compared to co-administration of both celecoxib (a COX-2 inhibitor) and t-AUCB (Hwang et al., 2011). Furthermore, COX-2/sEH inhibition also suppresses tumor growth by inhibiting angiogenesis, suggesting that creating future sEHs that also contain the ability to inhibit COX may be desirable in the treatment of certain diseases (Zhang et al., 2014a).

There have been significant challenges faced in obtaining Food and Drug Administration approval for sEHIs evaluated in completed clinical trials (Table 2) (GlaxoSmithKline, 2014a, b). Despite the lack of serious adverse effects caused by sEHI administration, some compounds, most notably GSK2256294, are on hold due to the rigorous standards that must be met for drug development pursuit. However, sEHIs are still undergoing evaluation for use in other disorders such as subarachnoid hemorrhage (Oregon H and Science U, 2018) and diabetes mellitus, (Vanderbilt University Medical C, 2018). In addition, novel sEHIs may be discovered based on previously developed pharmacophore models, which have identified several new compounds from the Specs database capable of inhibiting sEH in extremely low concentrations.

Table 2
A summary of completed and ongoing clinical trials involving sEHIs. sEHI treatment displays promise in a variety of disorders.

<table>
<thead>
<tr>
<th>Clinical Trials Involving sEHs</th>
<th>Drug</th>
<th>Disease</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00847899 (Chen et al., 2012)</td>
<td>A98281</td>
<td>Hypertension/Impaired Glucose Intolerance</td>
<td>No officially published results</td>
</tr>
<tr>
<td>NCT00654966 (Tran et al., 2012)</td>
<td>AUDA</td>
<td>Heart Failure</td>
<td>AUDA reversed urontenin-II induced vsaocustincretion in heart failure patients</td>
</tr>
<tr>
<td>NCT01762774 (GlaxoSmithKline, 2014a)</td>
<td>GSK2256294</td>
<td>COPD</td>
<td>Reduction of high-density lipoprotein was observed after dose escalation in healthy males and male moderately obese smokers</td>
</tr>
<tr>
<td>NCT02065337 (GlaxoSmithKline, 2014b)</td>
<td>GSK2256294</td>
<td>Safety</td>
<td>No serious adverse events occurred with 10 mg oral dose in healthy adult and elderly males and females</td>
</tr>
<tr>
<td>NCT03318783 (Oregon H and Science U, 2018)</td>
<td>GSK2256294</td>
<td>Subarachnoid Hemorrhage</td>
<td>Recruiting</td>
</tr>
<tr>
<td>NCT03486223 (Vanderbilt University Medical C, 2018)</td>
<td>GSK2256294</td>
<td>Diabetes Mellitus and Metabolic Disorders</td>
<td>Recruiting</td>
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(Waltenberger et al., 2016). This technique may be the forefront of drug discovery concerning sEH inhibition in the coming years. Furthermore, company Eicosis is investigating the use of EC2506, a sEH, as a treatment for diabetic peripheral neuropathy in a phase Ia clinical trial beginning in 2019 (Grant #R44EO025598). New sEHIs may be discovered naturally as well. Urea-based sEHIs have recently been isolated from the African plant Pentadiplandra brazzeana and shown to act as analogues in a rat model (Kitamura et al., 2015). While some promising sEHIs are facing resistance in the receipt of FDA approval, other innovative sEHIs are working towards translation to CNS disorders and optimization to the human species, and this atmosphere is dynamic and evolving.

4. Inflammation and sEH

Inflammation is a biological response to an injury that attempts to minimize functional and structural damage relating to the insult. The response is characterized by the dilation of arterioles and capillaries, increased permeability of microvasculature, and the invasion of leukocytes into injured tissue (Wagner et al., 2017). While inflammation initially serves as a mechanism to resolve injuries, it becomes a problem (and turns into a chronic condition) if it is not halted after a certain period of time (Wagner et al., 2017). Inflammation is a hallmark of many conditions, many of which involve the CNS. When affecting the CNS, inflammation (referred to as neuroinflammation) involves the recruitment of leukocytes and lymphocytes to the CNS and the activation of glial cells (including microglia, the phagocytes of the brain) (39). In some pathogenic processes, such as in stroke and TBI, inflammation can also cause increased permeability of the BBB, allowing harmful blood products to enter the brain parenchyma (Prakash and Carmichael, 2015). Neuroinflammation is a major cause of secondary injury cascades, and it is primarily regulated by microglia and peripheral leukocytes (Hung et al., 2017). Once activated, microglia produce pro-inflammatory cytokines that result in cytotoxic effects if not regulated. This property of microglia makes them a prime contributor to neuroinflammation (Hung et al., 2017). Due to its widespread and detrimental effects, treatment strategies have been designed to prevent or reverse neuroinflammation (Hung et al., 2017).

sEHs may serve as therapeutic options when treating several disorders, some of which are neurodegenerative, due to their ability to prevent EET and other EpFA metabolism by sEH (Bianco et al., 2009). EETs are made from ARA in the CYP reaction (Hung et al., 2017; Davis et al., 2017). The ARA network produces many inflammatory mediators that are implicated in numerous diseases, making the pathway another target of therapeutic intervention (Meng et al., 2015). EETs have many roles in the body, but they are quickly broken down and inactivated by sEH, preventing them from utilizing their anti-inflammatory features (Morrissette and Hammock, 2013). If sEH is inhibited, EET levels can increase and exert anti-inflammatory actions. The EET increase is limited by a variety of other degradation pathways making it difficult to increase EpFA to undesirable levels. Indeed, metal cations are shown to decrease in the serum during systemic inflammation, and they are known natural endogenous inhibitors of sEH (Draper and Hammock, 1999b). This implies that sEH activity is increased during inflammation. In fact, the increase is so dramatic that sEH message, protein, and catalytic activity can be used as a marker of tissue inflammation (Matsumoto et al., 2014). Another way to decrease the concentration of sEH is through genetic deletion, which has been shown to reduce neuronal death, apoptosis, brain edema, and BBB permeability following TBI (Hung et al., 2017).

The exact method utilized by EETs to reduce inflammation is unknown, but it has been shown that in certain brain conditions, sEH increases in microglia while EETs and other EpFAs decrease. Microglia have a distinct role in neuroinflammation; they produce proinflammatory cytokines that cause neuronal damage and BBB disruption (Hung et al., 2017). If sEH is inhibited in microglia, EpFAs will resulting increase and contribute to microglia deactivation and enhanced neuronal survival (Hung et al., 2017). EETs and other EpFAs have also been able to inhibit the expression of vascular cell adhesion molecule-1 (VCAM-1), E-selectin, intercellular adhesion molecule 1 (ICAM-1) and the nuclear translocation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (Wagner et al., 2017). VCAM-1, E-selectin, and ICAM-1 are all cell adhesion molecules, so without them leukocytes and other immune system cells are unable to attach to injured tissue, and NF-kB helps regulate the immune response by upregulating enzymes that contribute to inflammation (Wagner et al., 2017). Leukocytes, one of the trademarks of inflammation, are reduced in the presence of EpFAs but the exact mechanism by which it occurs has not been fully elucidated (Iliff and Alkayed, 2009).

In neurological diseases such as ischemic stroke, epilepsy, Alzheimer’s disease (AD), PD, and TBI, rudimentary research has begun showing the positive effects of increased EET and other EpFA concentrations following sEH inhibition. In ischemic stroke, EpFAs are protective against cell death in a way independent of effects on CBF and may be able to decrease the infarct size (Wagner et al., 2017; Bianco et al., 2009; Matsumoto et al., 2014), complementing the vascular mechanism of EpFAs. Epilepsy patients may be treated pre or post seizure with the anti-inflammatory properties of EpFAs, as anti-convulsants are unable to prevent seizures (Wagner et al., 2017; Vito et al., 2014). Regarding AD and PD, EpFAs may be able to improve mitochondrial functioning and reduce neuroinflammation from oxidative stress (Wagner et al., 2017). In TBI, EpFAs have the potential to decrease brain edema due to their ability to decrease neuroinflammation (Hung et al., 2017). sEHs’ ability to mount multi-pronged therapeutic processes appeals to complex injuries, such as stroke and TBI characterized by multiple cell death pathways, which likely cannot be arrested by a drug designed to target a singular degenerative process (Iliff and Alkayed, 2009).

The ability of sEHs to resolve inflammatory responses is promising, but more research is needed before they can be utilized clinically (Liu et al., 2009). sEH is present in most tissues, so the systemic use of inhibitors may have unintended effects outside of regulating inflammation (Bianco et al., 2009). Additionally, EETs and other EpFAs have been postulated to afford several functions in the brain, such as inhibiting inflammation, and releasing peptide hormones, but the exact pathway that EETs use in the brain remains poorly understood (Bianco et al., 2009). The specific mechanism of sEHs is also unknown, though the inflammation-associated P38-MAPK is a potential target (Hung et al., 2017). Much of the current literature involving sEH and sEHIs is focused on cardiovascular and renal conditions. The inflammatory responses in these conditions differ from neurodegenerative diseases due to the presence of the blood brain barrier and a variety of CNS specific cells. Before sEHs can be translated to a clinical setting for treating CNS disorders, additional studies are warranted to reveal how these molecules directly affect the brain and if unintended side effects are produced.

5. Stroke and sEH

Stroke, characterized by a loss of blood flow to the brain, remains a leading cause of disability and death globally, with approximately 800,000 stroke victims suffering each year in the United States alone (Benjamin et al., 2017). The extent of ischemic damage depends on the severity of CBF reduction, which determines the extent of oxygen and glucose deprivation from the cells. This hypoxic environment results in extensive apoptosis and necrosis (Mehta et al., 2007). Subsequent activation of the tissue’s immune response initiates the upregulation of pro-inflammatory cytokines (Chamorro et al., 2012), and a compromised BBB allows for the rapid influx of neutrophils, macrophages, leukocytes, B cells, and T cells to the stroke-damaged brain, further exacerbating neurodegeneration and aggravating the injury (Emsley et al., 2003).
Despite the prevalence of stroke, treatment options are severely limited. Thrombolytic therapy using tissue plasminogen activator (tPA) remains the only treatment currently approved by the Food and Drug Administration, yet only 3–5% of stroke patients qualify for this therapy due to strict requirements and a narrow administrative window (Adesoye et al., 2011). Attempts for therapeutic options for ischemic stroke have largely failed in lab-to-clinic translation, possibly owing to a narrow targeting approach to therapy. Focusing on a single molecular pathway among the complex ischemic cascade seems to be a misguided treatment strategy. An alternative therapeutic approach focusing on an agent that targets multiple mechanisms across the ischemic process may prove more effective (Iliff and Alkayed, 2009). By reducing endoplasmic reticulum stress and its sequelae, sEH harnesses this approach, and thus has emerged as a potential target for stroke treatment.

The use of sEH in stroke therapy is based on early genetic studies deciphering the connection between EPHX2 polymorphisms and susceptibility to cerebral ischemia. The Glu470Gly variant, corresponding to a narrow targeting approach to therapy. Focusing on a single molecular pathway among the complex ischemic cascade seems to be a misguided treatment strategy. An alternative therapeutic approach focusing on an agent that targets multiple mechanisms across the ischemic process may prove more effective (Iliff and Alkayed, 2009). By reducing endoplasmic reticulum stress and its sequelae, sEH harnesses this approach, and thus has emerged as a potential target for stroke treatment.

The broad therapeutic potential of sEHIs may arise from the multifaceted role of EETs on cardiovascular and cerebrovascular function. An upregulation of EET signaling following transient ischemia may serve as a preconditioning stimulus to protect the brain against future damage (Alkayed et al., 2002). These findings, along with the complex protective effects of ETTs, suggest that this signaling pathway is activated by ischemic trauma to provide neuroprotection. To this end, both chemical sEH inhibition and gene deletion have been largely successful at providing neuroprotection and reducing the infarct size following ischemic stroke. Even a single low-dose administration of the t-AUCB at the time of MCAO reduces infarct volume, improves behavioral outcome as assessed on various models varying in ages and sex to confirm the protective effects of sEHIs in treating ischemic stroke, there are still significant gaps in knowledge that must be examined for hopes of bringing this prospective therapy to the clinic. As discussed previously, the cellular and biological mechanisms underlying sEH neuroprotection in stroke remain unclear and warrant investigation. Future studies should clarify the differential effects between acute chemical sEH and EPHX2 gene deletion on ischemic mechanisms such as CBF regulation and inflammation. Moreover, chronic sEHIs are applied to cultured astrocytes following oxygen-glucose deprivation also increase levels of EETs and vascular endothelial growth factor (VEGF), indicating the significant role of astrocytes as possibly mediating sEHI-induced neuroprotection (Zhang et al., 2017). Furthermore, greater ischemic damage is correlated with increased levels of sEH mRNA and less EET production in the mouse endothelium after exposure to oxygen-glucose deprivation (Shah et al., 2012). Despite these documented efficacy data, the precise mechanisms of sEHIs as multi-target protection against ischemic injury remain unclear. Further studies are needed in the field to examine the broad effects of sEHIs on stroke models in vivo and to elucidate the factors contributing to this ischemic protection.

Because EETs are well known vasodilators (Iliff et al., 2009), a potential mechanism of ischemic protection by sEHs may be through the preservation of CBF. In contrast, the reduction of infarct volume by AUDA-BE in ischemic mice reveals no change in regional CBF rates (Zhang et al., 2007). While AUDA-treated rats display significantly smaller infarct size, AUDA produces no significant effect on vascular structure and acts independently of blood pressure changes (Dorrance et al., 2005). Interestingly, administration of t-AUCB at time of MCAO promotes a nonsignificant trend post-stroke towards enhanced CBF in the reduced infarct region, but does not significantly alter CBF (Shaik et al., 2013). Compounding this observation, sEH knockout (SEHKO) mice, with targeted EPHX2 gene deletion, show significantly higher rates of CBF, suggesting sEHIs may operate via vascular mechanisms (Zhang et al., 2008b). Conversely, overexpression of sEH results in an impairment of the vasodilatory effects of sEHIs and a larger infarct size in mice (Zhang et al., 2013a). The explanation for this difference between chemical sEH and EPHX2 gene deletion remains unknown, although the degree of inhibition appears as a contributing factor, in that chemical sEH inhibition may not be sufficient to enhance CBF compared to chronic loss of activity from gene deletion. This gap in knowledge warrants future investigation into the various degrees of inducing sEH inhibition, specifically focusing on CBF changes both regionally and collaterally. Clarifying the underlying vascular mechanisms of sEHs is crucial to understanding the profile of sEH and their capacity as therapeutic targets.

Equally relevant to the regenerative capacity of sEHIs pertains to the drug’s protective capabilities in models that closely approximate the comorbid elements of stroke, such as hypertension. Following MCAO, the resulting infarct volume is larger in spontaneously hypertensive stroke-prone rats (SHSPR) than in Wistar Kyoto (WKY) rats (Coyle and Jokelainen, 1983), making these models relevant targets for sEH inhibition therapy. AUDA still reduces infarct volume in SHSPRs following MCAO, but this protection operates independently of vascular mechanisms (Dorrance et al., 2005). Chronic sEH treatment provides vascular protection through reduced blood pressure and increases microvessel density in SHSPR but not WKY rats, and generates neural protection in both species (Simpkins et al., 2009). The varying neuroprotection of sEHIs on both SHRSP and WKY rats suggests its broad therapeutic potential, applicable to a wide profile of models representative of the human population.

While current studies suggest the neuroprotective potential of sEHIs in treating ischemic stroke, there are still significant gaps in knowledge that must be examined for hopes of bringing this prospective therapy to the clinic. As discussed previously, the cellular and biological mechanisms underlying sEH neuroprotection in stroke remain unclear and warrant investigation. Future studies should clarify the differential effects between acute chemical sEH and EPHX2 gene deletion on ischemic mechanisms such as CBF regulation and inflammation. Moreover, chronic sEHIs are significantly lacking which are needed to further shed light on the effects of various degrees of inhibition in stroke models. Additionally, future studies should investigate the optimal timeline for sEH inhibition, as the progress of ischemic injury involves complex time-dependent processes such as the inflammatory response. Despite the significant role of inflammation in exacerbating ischemic damage, studies investigating the anti-inflammatory mechanisms of sEHIs are severely limited. Of note, the administration of an sEH inhibitor (TIPU) following focal ischemia at reperfusion and 24 h later reduces infarct size by 50% and suppresses inflammation, as measured by a decrease of IL-1β by 3.5 fold and tumor necrosis factor-alpha by 2.2 fold (Tu et al., 2018). A decrease in infarct volume is achieved when sEH is administered before and during MCAO, as well as during reperfusion, likely by targeting the peak of the inflammatory response exacerbating stroke damage. The effects of sEH administration during post-ischemic reperfusion must be further examined on various models varying in ages and sex to confirm neuroprotective effects across a wider patient pool.

Evaluating various models in exploring the neuroprotective benefits of sEHIs remains critical to establish clinical significance. Sex differences pertaining to the distribution of sEH in the body have implications in the field of stroke. Interestingly, SEHKO female mice do not exhibit reduced infarct volume consistent with gene deletion in male models, possibly due to decreased sEH expression (Zhang et al., 2009). These findings suggest sexually dimorphic expression of sEH might explain in part the underlying differences in CBF and ischemic damage.
experienced between males and females. Along this line of investigation, sEH gene deletion, but not the t-AUCB, reduces infarct size in reproductively senescent female mice, yet neither method decreases infarct size in young mice (Zuloaga et al., 2014). This indicates that sEH protection against cerebral ischemia may be altered by age and gender. Since in mice, sEH levels are profoundly influenced by both, the lack of data evaluating female and/or aged models in sEH inhibition for ischemic stroke should direct future research to investigate the effects of this inhibition in various models emulating a representative stroke population. Recognizing the therapeutic potential of sEHIs involves a multi-target approach to ischemic stroke suggests that the complexity of this therapy will require further research to clarify the factors of sEH-mediated neuroprotection.

6. Traumatic brain injury and sEH

Operating under similar mechanisms as in stroke, sEH as a therapeutic target for traumatic brain injury (TBI) is much less documented. TBI affects unique groups of people in disproportionate rates, such as military personnel and incarcerated individuals. About 12% of military personnel (Schneiderman et al., 2008) and 60–25% of incarcerated persons (Shirama et al., 2012) have a history of TBI. TBI, characterized by a blow or penetration to the head, induces primary damage to the brain tissue, followed by cerebral inflammation representing a major secondary cell death pathway (McConeghy et al., 2012). EET and other EpFA levels in the brain increase following TBI (Boezaart and Kadieva, 1992; Hung et al., 2017). Exogenous administration of 14,15-EET and AUDA promotes angiogenesis, reduces neuroinflammation, suppresses astrogliosis, and improves behavioral outcome in ischemic stroke models (Liu et al., 2016). Moreover, administration of TTPU significantly reduces inflammation, as evaluated by a decrease of pro-inflammatory cytokine TNF-α by 2.2 fold and of IL-1β by 3.5 fold (Tu et al., 2019). This capacity of sEHIs to attenuate inflammation and promote neuroprotection suggests the therapeutic promise of sEHIs in treating TBI in addition to stroke. Targeting sEH in TBI models reveals that SEHKO mice outperform wild-type mice on the beam walk, indicating improved motor coordination both pre- and post-TBI (Strauss et al., 2013). Interestingly, SEHKO mice exhibit minor learning deficits in the Morris water maze independent of TBI (Strauss et al., 2013). Sham-operated SEHKO mice also express an unexpected injured phenotype in the water maze assessing working memory, resembling similar deficits as brain-injured mice (Strauss et al., 2013). A valid consideration for these unexpected findings is the role of the EPHX2 gene in both epoxide hydrolase and lipid phosphatase activity (Newman et al., 2003), possibly explaining the impaired behavioral phenotypes associated with SEHKO mice. Further studies investigating SEHKO mice and selective sEH inhibition are needed to confirm the role of sEH in memory consolidation and motor coordination following TBI. Examining discrete brain regions (e.g., hippocampus) to evaluate memory deficits commonly associated with TBI would enhance our knowledge of the broader potential of sEHIs. Moreover, gene deletion of sEH significantly ameliorates TBI-induced brain tissue damage and neurological deficits, as shown by reduced neuronal cell death, brain edema, BBB permeability, apoptosis, matrix metalloproteinase 9 activity, and neurophil infiltration (Hung et al., 2017). SEHKO mice also exhibit an increase in anti-inflammatory M2 microglia/macrophages and a decrease pro-inflammatory M1 microglia/macrophages in the injured cortex, as well reduced levels of cytokines IL-1β, IL-6, MIP-2, and MCP-1, suggesting the anti-inflammatory effect of sEH deletion in TBI (Hung et al., 2017). Pharmacological inhibition by AUDA also attenuates neuroinflammation, brain edema, and apoptosis following TBI (Hung et al., 2017). Both sEH gene deletion and pharmacological inhibition by sEHIs indicate functional benefits of targeting sEH in TBI. While these limited studies suggest the neuroprotective and anti-inflammatory potential of sEH inhibition, significant gaps in knowledge must be addressed to validate its therapeutic promise for TBI. Although gene deletion of EPHX2 shows significant promise in attenuating TBI-induced functional and neurological deficits, discrepant results are observed between SEHKO individuals and chemical sEHIs. In stroke models, t-AUCB administration at time of MCAO does not significantly alter CBF (Shaik et al., 2013), while SEHKO mice show markedly higher CBF rates (Zhang et al., 2008b). Moreover, gene deletion of sEH, but not t-AUCB, reduces infarct size in female mice following stroke (Zuloaga et al., 2014). The challenges observed in sEH stroke models are of interest in TBI due to the overlap of injury mechanism. This warrants the need for investigating the effects of chemical sEH administration and sEH gene deletion on neurodegeneration and inflammation in TBI models, to better understand the optimal extent of inhibition. The appropriate dosage of sEHIs also represents an area of future direction because of the profile of patient suffering from TBI. The incidence of TBI is especially prevalent in athletes (Tagge et al., 2018) and military combat settings, with more than 300,000 US service members since 2000 sustaining a TBI (Helmick et al., 2015). These TBI victims represent a younger and healthier patient pool compared to those suffering from stroke, given the pertinent association to co-morbidity factors such as aging and obesity. Therefore, the appropriate sEH therapeutic threshold dosages may be lower for TBI patients than stroke, but still achieve a better prognosis. In the same vein, while stroke and TBI are highly unpredictable diseases, the occurrence of TBI in soldiers and athletes is likely higher than at-risk stroke patients, suggesting a unique opportunity for prophylactic sEHIs for TBI. Additionally, penetrating brain injury (PBI), comprising all TBIs which are not caused by a blunt mechanism, represents a new area of future direction for sEH therapy. Treatment for such open head wounds have shifted from aggressive removal of deep fragments to antibiotic prophylactic treatment to improve neurological outcomes (Kazim et al., 2011). This represents an underexplored opportunity for delivery of sEHIs to prevent subsequent damage by anti-inflammatory and neuroprotective mechanisms. Although inhibition of sEH is gaining ground in the field of TBI therapy, further research is needed to ascertain the therapeutic potential of these inhibitors.

7. Parkinson’s disease and sEH

PD is one of the most prevalent neurodegenerative diseases, second only to AD (Ren et al., 2018). Within the next 20 years, the number of patients with PD is predicted to double (Chen, 2010). PD is responsible for the progressive deterioration of normal movement and balance capabilities along with other nonmotor symptoms (Chen, 2010). Over the course of PD, dopaminergic neurons of the substantia nigra die prematurely and Lewy bodies (abnormal protein aggregates of α-synuclein) accumulate in the brain (Kalia and Lang, 2015). Both motor deficits characteristic of PD including speech impediments, dyskinesia, and nonmotor impairments such as psychosis are unable to be sufficiently treated with current medical or surgical approaches (Kalia and Lang, 2015). Current medical options attempt to treat PD by increasing dopamine (DA) concentrations or through the stimulation of DA receptors. These approaches are likely not satisfactory due to their limited targeting of the basal ganglia and DA as opposed to a wider range of the neurotransmitters involved in PD (Kalia and Lang, 2015). Other nonpharmacological treatment options such as physiotherapy are being examined; however, these preliminary findings have yet to yield accepted treatment options in humans (Bloom et al., 2015). Though certain treatments may alleviate symptoms of PD, there is currently no cure and the trigger for the brain death characteristic of the disease is not well understood, restricting treatment to the later phases of the disease (Borlongan, 2018).

sEH levels are elevated in the regions of the brain that correlate with dopaminergic death in both humans and animal models of PD (Ren et al., 2018). The expression of sEH is also indicative of striatal α-synuclein phosphorylation (Ren et al., 2018). Because PD may not display symptoms until up to 80% of striatal DA has already been lost, a means
for earlier diagnosis would be highly progressive in broadening the search for treatment options (Marsden, 1990). Peripheral detection of elevated levels of sEH in the gut may be possible prior to dopaminergic death, potentially providing an early indicator of PD (Borlongan, 2018; Marsden, 1990).

The detrimental effects of sEH are well documented (Ren et al., 2018; Qiu et al., 2015). In a mouse model of PD, both a deficiency in sEH and the administration of sEH substrate, 14,15-EET, reduces tyrosine hydroxylase-positive cell death (Qiu et al., 2015). The deletion of the sEH gene also protects against neurotoxicity in a PD mouse model (Ren et al., 2018). Although the inhibition of this gene does not provide a current treatment approach for PD patients, it does allow future medical therapies to target this enzyme as method for slowing the progression of PD.

Furthermore, the inhibition of sEH may also provide a treatment for PD (Ren et al., 2018). The clinical utility of sEH inhibition is recognized in various heart and lung diseases in asthma patients and smokers, as well as in the brain, discussed here (Ono et al., 2014; Yang et al., 2017a). sEH inhibition likely reduces both oxidative stress and neuroinflammation through the elevation of endogenous EETs and other EpFAs (Qiu et al., 2015; Lakkappa et al., 2018). An inhibitor of sEH and COX-2, TPUPB provides significant neuroprotective activity in a model of PD using Drosophila melanogaster (Lakkappa et al., 2018). TPUPB, another inhibitor of sEH, significantly alleviates the reduction of DA, 3,4-dihydroxyphenylacetic acid and homovanillic acid post-MPTP-induced neurotoxicity in a mouse model (Ren et al., 2016, 2018). Because the degeneration of dopaminergic neurons is one of the main characteristics of PD, sEH could ameliorate the progression of the disease (Kalia and Lang, 2015). In tandem, its potential to slow PD progression suggests that TPUPB would likely be more effective if its administration began earlier in the course of the disease, prior to the onset of symptoms. Future trials examine the efficacy of such sEH inhibition treatments beginning at various stages of PD would guide the translational application of this drug to the clinic. The practical benefits of TPUPB indicate that the repeated oral delivery of TPUPB significantly reduces neurotoxicity in the striatum of mice (Ren et al., 2018). Indeed, orally administered TPUPP demonstrates the ability to suppress apoptosis in PARK2 neurons, further demonstrating the negative effects of sEH and the benefits of its inhibition (Ren et al., 2018). As noted above, EETs have previously been used in cases of cerebral stroke, cardiac failure, and hypertension to ameliorate inflammation, caspase activation, and apoptosis, among other damaging processes (Lakkappa et al., 2016). Inhibition of sEH has the potential to increase the half-life of endogenous EETs. EETs may aid in neuroprotection in models of PD through mechanisms similar to their cytoprotective properties in models of other disorders such as stroke, TBI, and epilepsy (discussed later) (Lakkappa et al., 2016). Inhibition of sEH in PD has yet to be clinically verified. In vivo animal models lend support to both the significant detrimental implications of elevated sEH levels and the ameliorating effect of reducing the expression levels of the enzyme. It is critical to further study the use of elevated sEH levels in the brain as a biomarker of early PD detection and its treatment with sEH inhibition prior to onset of symptomatic loss of striatal DA and neuronal death. Moreover, the use of sEH inhibitors such as TPUPP and EETs should be evaluated at different stages of PD in animal models and clinical trials to determine whether they are viable as treatment options for patients with preexisting and progressive stages of PD.

8. Other neurological disorders and sEH

8.1. Epilepsy

In addition to stroke, PD, and TBI, sEH has been implicated in epilepsy within the last five years. Epilepsy is defined as recurrent seizures characterized by extensive, synchronized excitatory signaling by neurons (Stafstrom and Carmant, 2015). Neuroinflammation, paramount to the disease process in other CNS disorders, has been linked to epileptic pathogenesis (Vezzani et al., 2013; D’Ambrosio et al., 2013). Due to its role in inflammation, sEH has become an attractive target for therapeutic intervention in epilepsy. AUDA treatment in rats subjected to induced temporal lobe status epilepticus and the inflammatory response decreases expression of inflammatory cytokines IL-1β and IL-6 in the hippocampus (Hung et al., 2015). The ratio of EETs to DHETs is also increased in the AUDA treated group, suggesting that the inhibition of sEH slowed the degradation of EETs. Notably, treatment with AUDA successfully increases seizure induction threshold, and decreases seizure susceptibility (Hung et al., 2015). This observation suggests that inhibition of sEH produces therapeutic, anti-inflammatory effects in the brain as well as in peripheral systems.

Although inflammation is key to epilepsy pathology, other findings suggest that sEHs may confer protection via other mechanisms. Mice administered sEHs and exogenous EETs are resistant to gamma-amino butyric acid (GABA) antagonist seizure induction (Inceoglu et al., 2013). It is hypothesized that CYP is involved with GABA signaling, which is a key epileptic pathway (Inceoglu et al., 2013). However, these mice remain susceptible to seizures induced by other means, as evidenced by the failure of sEHI 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS) to halt seizures produced by powerful convulsant tetramethylenedisulfotetramine (TETS) when administered after the seizure onset, but is effective when initiated prior to the seizure (Vito et al., 2014). In addition, TUPS is effective at reducing TETS-induced cell death and neuroinflammation when administered in combination with GABA-A receptor agonist dizapam (Vito et al., 2014). These results suggest that EETs offer therapeutic ability by modulating excitatory signaling in addition to attenuating inflammation. EETs’ effects on neuronal excitability are supported by an additional finding that EET isomer 11,12 EET hyperpolarizes CA1 pyramidal neurons in the hippocampus by inducing increased G-protein-activated K+ conductance (Mule et al., 2017).

The applications of sEHs in epilepsy hold promise, but clinical translation is lacking. In a study of 20 patients with temporal lobe epilepsy, sEH enzyme levels are increased compared to the control group (Ahmedov et al., 2017). Furthermore, seizure duration and frequency correlate with sEH level (Ahmedov et al., 2017). This suggests that there is a niche for sEH intervention in humans, but more studies must be conducted to better elucidate the role of sEH in epilepsy.

8.2. Cognitive impairment and dementia

Pathologic manifestations of cognitive decline and dementia implicate sEH’s important role in these debilitating neurodegenerative diseases. An estimated 13.9% of people over the age of 71 suffer from the cognitive decline, making it a major public health concern (Plassman et al., 2007). Causes of dementia include AD, vascular pathology, accumulation of Lewy bodies, and others. Based on the documented pathological effects of EETs on vasculature, sEH has been identified as a potential therapeutic target for vascular cognitive decline. sEH has also been recently identified to have detrimental consequences in AD, and the relevant research will be discussed below.

Accumulation of beta amyloid protein aggregates (Aβ) is a hallmark of AD and a strong initiator of cytotoxicity. Recently, a pathological link between Aβ and the formation of epoxides from CYP P450 is detected in cerebral microsomes prepared from brain tissues of Sprague-Dawley rats that show a 30% decrease in 14,15 EET production when cultured with Aβ. These findings suggest that EpFA production is impaired in AD (Sarkar et al., 2011). While limited data support the relationship between sEH and AD, this study serves as a platform for future investigation.

The role of sEH in vascular cognitive impairment (VCI) is notable in individuals diagnosed with VCI that showed increased DHET levels in brain tissues post-mortem, suggesting upregulated activity of sEH (Nelson et al., 2014). Moreover, increased sEH immunoreactivity in the
cerebrovascular endothelium is observed adjacent to hyperintense white matter lesions (WMH), which are characteristic of VCI. The R287Q sEH polymorphism is also associated with increased WMH volume (Nelson et al., 2014). Interestingly, there is an association between WMH and psychiatric disorders that involve cognitive impairment (Swadfager et al., 2018). This implies that there is an intimate relationship between cell signaling, cerebrovasculature, and behavior, and that sEH may represent a common denominator when investigating possible treatment options for these disorders. These results suggest that sEH may represent a viable target for the treatment of cognitive impairment in humans, though more in vivo and in vitro research must be conducted to establish a causative relationship.

8.3. Depression

Depression is the most common psychiatric disorder, and can cause crippling symptoms such as fatigue, suicidality, lack of appetite, and anhedonia. To date, treatment options for depression are relatively limited to selective-serotonin reuptake inhibitors (SSRIs), selective-norepinephrine reuptake inhibitors (SNRIs), and counseling (Hashimoto, 2016). Even with the availability of SSRIs and SNRIs, many still do not respond to pharmacological intervention. Inflammation, like in other diseases affecting the brain, could have a feasible and damaging role in depression as well, as suggested by Zunszain, Heppul, and Pariante (Zunszain et al., 2013). This paves the way for the notion that EETs may have advantageous effects on depressive symptoms due to their anti-inflammatory properties.

Seasonal major depression is accompanied by increased sEH derived oxylipins in patients during the winter, suggesting increased sEH activity (Hennebelle et al., 2017). Although the clinical significance of this study is limited by the small sample size (n = 15), these results offer venues for more studies investigating the use of sEH derived oxylipins as potential biomarkers for depression. Furthermore, TPPU administration is associated with ameliorated depressive symptoms in social defeat stress models in mice (Ren et al., 2016). SEHKO mice and TPPU-treated mice also display greater resilience to social defeat stress, which corresponds to upregulated brain-derived neurotrophic factor signaling via the TrkB receptor (Ren et al., 2016), (Wu et al., 2017). This provides further evidence for sEH inhibition’s integral role in depression pathology and its potential use as an antidepressant. More studies must be executed to uncover the exact mechanisms by which sEH is involved in depression.

8.4. Subarachnoid hemorrhage

Subarachnoid hemorrhage has a similar mechanism of injury as the disorders mentioned above, with acute inflammation serving as a major bad actor in poor outcomes (Miller et al., 2014). Therefore, sequestrates of the inflammatory response in subarachnoid hemorrhage may be beneficial, and this may be mediated by an increased level of EETs. In SEHKO mice, less edema is observed in white matter tracts after subarachnoid hemorrhage-induction compared to wild-type mice (Siler et al., 2015a). SEHKO mice are also found to express less VCAM-1 after subarachnoid hemorrhage, which is a marker of damaged endothelium (Siler et al., 2015a). This may be the reason for the reduction in edema in these mice. 14,15-EET is also shown to be protective against delayed cerebral ischemia in mice subjected to subarachnoid hemorrhage (Siler et al., 2015b). As described above, there is one clinical trial that has been launched, aiming to evaluate the effects of GSK2256294 (Oregon H and Science U, 2018). Although the use of sEHs as treatment for subarachnoid hemorrhage is still in its rudimentary phase of clinical evaluation, preclinical data are supportive.

9. Obesity and sEH

Obesity is an increasingly common public health problem, particularly in westernized countries with poor diets consisting of simple carbohydrates and trans-fats (Blias and Whiteside, 2018). The estimated prevalence of obesity in the United States is 36.5% of adults (Ogden et al., 2015). Obesity is a major risk factor for a variety of severe health conditions such as diabetes mellitus and cancer due to an increased chronic inflammatory state (Garg et al., 2014). While there are many factors influencing weight, such as diet, genetics, and physical activity, the brain-gut axis plays an especially important role in endocrine signaling and microbiome interaction (Bliss and Whiteside, 2018). sEH inhibition represents a promising treatment for cardiovascular diseases, discussed in detail above. There is accumulating evidence that sEH may contribute to obesity-related disorders as type II diabetes mellitus, hypertension, and cardiomyopathy (Huang et al., 2016).

In rats, there is an observed post-prandial decrease in sEH, marked by oxypolin (Yang et al., 2017b). This decrease in sEH activity is additive with increased dietary potassium, which is shown to decrease risk of stroke (Yang et al., 2017b; D’Elia et al., 2011). However, rats treated with antibiotics to eradicate gut flora do not show the attenuation of sEH activity seen in non-treated rats (Yang et al., 2017b). This information suggests that sufficient dietary potassium may induce inhibition of sEH modulated by the gut microbiome, and altogether may be protective against cerebrovascular accident. In rats fed a high-fat diet for 10 weeks, CYP-derived EET production is significantly decreased compared to rats with a normal diet (Wang et al., 2003). Mice treated with sEHIs for 5 weeks demonstrate a 32% decrease in caloric intake after being fed a high-fat-high-fructose diet prior to the sEH administration, leading to weight loss (do Carmo et al., 2012). The same mice also show decreased leptin levels after sEH treatment (do Carmo et al., 2012). Furthermore, colonic inflammation associated with obesity is correlated with increased levels of sEH in mice, and its genetic deletion is associated with decreased inflammation (Wang et al., 2018). Therefore, diet, a major contributing factor to obesity, is shown to influence the production of EETs and activity of sEH. As discussed thoroughly above, increased availability of EETs/sEH inhibition may be protective in stroke. The established interaction between the gut microbiome (Galland, 2014) and brain, due to the findings that sEH activity may be partially dependent on gut bacteria modulation, may be of interest in future research concerning sEH in obesity and obesity-related disorders. In summary, sEH inhibition may promote a healthy gut, which not only has beneficial effects on the gastrointestinal tract and obesity, but also on the brain.

10. sEH therapeutic effects in other non-CNS organs

sEHIs exhibit therapeutic effects in other non-CNS organs, such as the liver, heart, and kidney (Harris et al., 2015; Sirish et al., 2013; Kim et al., 2014). Particularly, sEHIs mitigate tissue fibrosis in several organs such as the liver, as TPPU reduces collagen deposition, inflammation, and endoplasmic reticulum stress in the livers of mice given carbon tetrachloride (Harris et al., 2015). TPPU also attenuates heart remodeling associated with cardiac fibrosis by improving heart function, decreasing collagen deposition, reducing cardiac fibroblasts, and preventing cardiomyocyte hypertrophy (Sirish et al., 2013). Additionally, sEHIs reduce myocardial infarct sizes after heart ischemia and preclude cardiac hypertrophy and arrhythmia via EET-related pathways in animal models (Gross et al., 2008; Seubert et al., 2006). Similarly, inhibiting sEH decreases inflammation, cell death, and oxidative stress, and precludes renal fibrosis in mouse kidneys (Kim et al., 2014). Moreover, sEHIs protect against hypertension-related damage to vascular and glomerular structures in the kidney and reduce collagen type IV expression (Zhao et al., 2004; Imig et al., 2012). In fact, administering AUDA prevents hypertension and type 2 diabetes-associated renal damage by decreasing urinary albumin and MCP-1 excretion and the infiltration of monocytes/macrophages in the kidney (Olearczyk et al., 2009). Thus, sEHs are capable of protecting the heart and kidney from cardiovascular diseases such as hypertension (Imig
and Hammock, 2009). Furthermore, sEHIs’ anti-inflammatory properties and ability to mitigate systemic inflammation (Schmelzer et al., 2005) may also prevent additional inflammation-induced damage to these organs resulting from the actions of pro-inflammatory molecules and pathways following tissue insult (Harris et al., 2008).

11. Stem cells and sEH

The anti-inflammatory properties of sEHIs in CNS disorders can be enhanced by similar anti-inflammatory and regenerative characteristics of stem cell therapy. Although the mechanisms of stem cell therapeutic techniques remain not well understood, even short-lived stem cell engraftment may serve as key regenerative process towards direct replacement for damaged or dead cells (Rolfe and Sun, 2015; Borlongan et al., 2004; Lee et al., 2017). A concern of sEHI use is that it may reduce endogenous stem cell proliferation (Fromel et al., 2012), and this may be amended through the engraftment of exogenous stem cells to maintain neurogenesis. Additionally, transplanted stem cells can provide indirect aid by secreting a variety of therapeutic molecules to stimulate the repair of dying brain cells (Blondini et al., 2010; Park et al., 2008; Lee et al., 2017) with anti-inflammatory factors among these secreted factors as highly potent for affording regeneration (Chen et al., 2003; Drago et al., 2013; Blais et al., 2013). Similarly, sEHIs exhibit anti-inflammatory properties (Harris and Hammock, 2013; Liu et al., 2005), making them prime candidates for combination therapy with stem cells. In tandem, stem cells can advance sEHI applications by compensating for the deteriorating endogenous cells through reduction of inflammation-plagued CNS disorders.

sEH overexpression in CNS disorders characterized by protein aggregate-mediated inflammation may benefit from a combination of sEHI inhibition and stem cell therapy. In PD patients, sEH levels are elevated and centralized around inflamed areas associated with α-synuclein aggregation (Borlongan, 2018), a pathological hallmark of the disease. While α-synuclein aggregation in PD is primarily observed in the brainstem and cortex, such aberrant protein accumulation can also be detected peripherally. Thus, sEH, a mediator in the accumulation of α-synuclein, may be detected outside the CNS as well. If altered sEH levels can be assayed peripherally, then early diagnosis and intervention are possible to prevent extensive dopaminergic neuron loss in PD, whose symptoms are not recognized until 80% of the dopaminergic neurons are depleted. Interestingly, sEHI inhibition reduces neurotoxicity in cell and animal models (Ren et al., 2018), and the treatment may be enhanced by the addition of stem cells (Borlongan, 2018). While sEH activity in plasma is very low, the activity and protein can be monitored in peripheral blood cells. Because endogenous stem cells from PD patients show increased concentrations of sEH, cell replacement with exogenous stem cells containing lower levels of sEH represents a viable method for promoting brain regeneration (Borlongan, 2018). Interestingly, similar to PD, a pathological link between inflammation and protein aggregation accompanies other CNS disorders; notably mutant huntingtin in HD (Luo et al., 2018), platelet-leukocyte aggregations in stroke (Tao et al., 2016), increased α-synuclein in TBI (Acosta et al., 2015a), increased amyloid and tau in dementia (Feminella et al., 2018), depositions prion protein upregulation in clinical depression (Beckman and Linden, 2016), and spectrin aggregate formations in epilepsy (Syrbe et al., 2017). Cognizant of these overlapping pathologies, combination therapy of stem cells and sEH stands as potentially efficacious strategy to treat CNS disorders plagued by neuroinflammation and proteinopathy.

sEH inhibition may harbor an anti-inflammatory environment in CNS disorders due to the intrinsic properties of EETs (Zhang et al., 2007; Harris and Hammock, 2013; Yang et al., 2015b), and this therapeutic pathway may be enhanced by stem cell adjunctive therapy (Fig. 3). Indeed, sEHIs have been studied in the context of stroke (Zhang et al., 2007; Shaik et al., 2013; Shen and Hammock, 2012), epilepsy (Inceoglu et al., 2013), PD (Lakkappa et al., 2018), and TBI (Hung et al., 2017), which are well-documented disorders with neuroinflammation as a major secondary cause of cell death. In similar fashion, stem cell therapy in these disorders dampens inflammation (Neal et al., 2018; Stonestifer et al., 2017; Acosta et al., 2015b; Lozano et al., 2015; Acosta et al., 2014; Lippert et al., 2018; Rao et al., 2017). sEHIs suppress inflammation by reducing proinflammatory lipid mediators (Schmelzer et al., 2005), but an equally potent anti-inflammatory mechanism is the activation of the EET-induced PPAR-γ pathway (Liu et al., 2005), which inhibits the NF-κB pathway and reduces VCAM-1 expression (Morisseau and Hammock, 2013; Liu et al., 2005; Shen and Hammock, 2012). Conversely, PPAR-α and PPAR-γ agonists are potent inducers of the sEHI enzyme (De Taeye et al., 2010). In parallel, the anti-inflammatory properties of stem cells (Stonestifer et al., 2017; Acosta et al., 2015b; Kim et al., 2010), acting likely via reduction of ICAM-1 is also seen in stem cell transplants in experimental stroke (Cheng et al., 2018). Altogether, the postulated mechanism mediating inflammation in CNS disorders reveals equally common cellular and molecular targets to probe stem cells and sEHI interactions. Furthermore, the recognition of sEHI-mediated inflammation and the poor stem cell survival following transplantation due to inflammation (Sullivan et al., 2015) offers novel insights for the use of sEHIs to enhance stem cell therapeutic effects.

sEHIs and stem cells share many signaling pathways, which may explain their similarities in the resulting functional outcomes. The Wnt and PI3K/AKT signaling pathways are involved in both sEH and stem cells (Fromel et al., 2012; Chen et al., 2013; Takahashi et al., 2005; Van Camp et al., 2014). Interestingly, both the PI3K/AKT and AKT/GSK3β pathways are regulated by EETs for promoting angiogenesis (Qu et al., 2015) and endothelial progenitor cell (EPC) function (Guo et al., 2018), respectively. In addition, the EET-mediated activation of the PI3K/AKT pathway provides protection after cerebral ischemia/reperfusion injury (Qu et al., 2015). With this in mind, the idea that sEHIs enhance stem cell therapeutic effects via PI3K/AKT or AKT/GSK3β pathways should be further explored to fully understand the benefits of sEHIs on stem cell survival towards optimizing an sEH-based stem cell combination therapy.

sEHIs and stem cells both enhance the vasculature via the PPAR-γ pathway, an established regulator of angiogenesis. EETs are endogenous activators of PPAR-γ (Morisseau and Hammock, 2013; Harris and Hammock, 2013; Liu et al., 2005), which can modulate EPC function (Xu et al., 2013; Guo et al., 2018). Moreover, sEHIs increase the expression of VEGF and hypoxia-inducible factor-1α (HIF-1α) via PPAR-γ (Xu et al., 2013), which are secreted by stem cells (Stonestifer et al., 2017) and are neuroprotective for CNS disorders (Greenberg and Jin, 2013). Furthermore, MSCs can synthesize EETs (Kim et al., 2010), and in turn EETs are able to enhance stem cell migration following transplantation (Li et al., 2015). Equally convincing evidence also show that sEH and EETs can inhibit hematopoietic progenitor cell proliferation and MSC-derived adipocyte stem cell differentiation (Fromel et al., 2012; Kim et al., 2010; Vanella et al., 2011), suggesting that a much more careful assessment is necessary when contemplating sEH inhibition and stem cell combination therapy. Along this cautionary note, both the NF-κB and Wnt signaling pathways are involved in cancer stem cells (Van Camp et al., 2014; Rinkenbaugh and Baldwin, 2016; Reya et al., 2001). Supporting this, sEH expression is found to be increased in many different neoplastic tissues (Harris and Hammock, 2013). Translational research efforts evaluating sEHIs’ role in reducing the potential tumorigenic risk of stem cell therapy while maximizing their therapeutic effects (Stonestifer et al., 2017; Reya et al., 2001) will guide not just the efficacy, but also the safety of sEH and stem cell combined therapy.

12. Synergistic effects of sEHIs combined with other compounds

While benefits of sEHIs are typically associated with EETs, which are anti-inflammatory and promote vasodilation (Imig and Hammock, 2009), other ΕPΦAs are also active and exert detrimental or beneficial
effects (Wagner et al., 2017). For instance, sEH metabolizes linoleic epoxides to linoleic diols, which demonstrate toxicity, modulate thermogenesis and brown adipose tissue, and cause vascular permeability and sepsis (Zhang et al., 2014b; Wagner et al., 2017). Epoxyeicosa-trienoic acids (EEQs) from eicosapentaenoic acid (EPA) are implicated in anti-inflammation and various organs such as the lungs and uterus (Morin et al., 2010). Epoxydocosapentaenoic acids (EDPs) from docosahexaenoic acid (DHA) are often more active than EETs, are highly present in the retina and brain (Zhang et al., 2014b), and possess potent anti-angiogenic (Zhang et al., 2013b) and vasodilatory (Zhang et al., 2014b) properties. In particular, 17,18-EEQ and 19,20-EDP decrease autophagy and ER stress in obese mice (Lopez-Vicario et al., 2015) and inhibit sEH metabolism more effectively than other omega-3 regioisomers (Zhang et al., 2014b). Interestingly, EEQs and EDPs may exhibit a greater increase in vasodilation and decrease in pain and inflammation than EETs (Morisseau et al., 2010). Of note, omega-3 rich diets promote anti-inflammation and raise plasma and tissue concentrations of EEQ and EDPs in mammals, as these compounds are derived from omega-3 fatty acids (Arnold et al., 2010). Moreover, sEHIs are more efficient in most assays if the animal’s diet is high in omega-3 fatty acids and lacks omega-6 fatty acids (Lopez-Vicario et al., 2015; Arnold et al., 2010). sEHIs exhibit beneficial synergistic effects such as increased anti-inflammation when applied with other compounds. Combining sEHIs with a diet rich in omega-3 polyunsaturated fatty acids (PUFAs) mitigates hypertension induced by angiotensin-II, increases EPA and DHA epoxides in the kidney, decreases inflammation, and inhibits COX and LOX metabolic pathways compared to a diet rich in PUFAs and no administration of sEHIs (Liu et al., 2013). Moreover, using sEHIs in tandem with nonsteroidal anti-inflammatory drugs (NSAIDs) exerts synergistic effects such as increased antinociception and reduced COX enzyme expression, which leads to decreased pain and inflammation while avoiding detrimental thrombotic cardiovascular side effects associated with NSAIDs (Schmelzer et al., 2006). Indeed, co-administration of aspirin and t-AUCB enables a lower dose of aspirin to be used, achieving the same anti-inflammatory benefits as a higher dose while minimizing the risk of blood clotting and gastrointestinal side effects (Liu et al., 2010). Administering the COX-2 inhibitor (coxib) celecoxib together with t-AUCB reduces inflammation, increases eicosanoids like EETs that provide cardiovascular protection, and inhibits tumor angiogenesis to preclude tumors from growing and metastasizing in animal cancer models (Zhang et al., 2014a). Similarly, t-AUCB boosts 5-lipoxygenase (5-LOX) activation protein (FLAP) inhibitor MK886-mediated anti-inflammation and lipoxygenase inhibition, apparent by the reduction in the pro-inflammatory 5-LOX metabolites such as leukotriene and 5-HETE in a murine model (Liu et al., 2010). Furthermore, adding omeprazole, a CYP 450 inducer, together with TPPU has a combined effect which increases pain reduction, P450 activity, and EET levels (Goswami et al., 2015). Combining PPAR-γ agonists and sEHIs reduces glomerular injury in the kidneys of hypertensive obese rats significantly more than the PPAR-γ agonist or sEH alone (Imig et al., 2012). Of note, administering both sEHIs and phosphodiesterase inhibitors has a stronger effect on decreasing pain and elevating the plasma ratio of epoxy/dihydroxy fatty acids (Inceoglu et al., 2011). With these favorable synergistic effects, it is possible that combining sEHIs and stem cells may augment the latter’s anti-inflammatory characteristics and may exhibit other beneficial effects.

Administration of both sEHIs and exogenous EETs have beneficial effects on the brain and other body systems, as described above.

Fig. 3. The potential mechanisms by which sEH inhibition and stem cell therapy may be synergistic. Beneficial properties of each treatment may be enhanced when used in combination, improving outcomes of CNS diseases.
Inhibition of sEH seems to be a more important objective than administering EETs themselves. EEQs and EPDs may have more anti-inflammatory properties than EETs, the availability of which would be increased by sEH inhibition and not by EET administration (Morisseau et al., 2010). The unique distribution of sEH in the brain also presents a therapeutic challenge. This supports the inhibition of sEH as a target, so that effects take place where the enzyme works, rather than the transplant of EETs. EETs are also susceptible to stomach acid degradation, beta oxidation, and hydroxylation (Fang et al., 2001). However, AUDA shows significantly more activity in the dilation of mesenteric vessels than other sEHs, and this has been attributed to its ability to mimic epoxides (EETs and EpFAs) (Olearczyk et al., 2006). Therefore, there is evidence that an ideal therapeutic regimen may entail the use of both EETs and sEHs.

13. Conclusion

sEH is an enzyme that has been linked to many pathological conditions within the last 20 years, ranging from cardiovascular disease to neurological disease. Its association with CNS disorders has been more recently established, and inhibition of sEH shows robust therapeutic potential. Some of the applications of sEHs include the attenuation of inflammation, regulation of blood flow, and protection of neurons from excitotoxicity. However, many more mechanisms could be uncovered and further investigation of sEHs involvement in CNS disorders is warranted. In addition to discovering other methods by which sEHs may be beneficial to the progression of neurological diseases, the pharmacological properties of such drugs must be optimized. The envisioned safe and effective sEH will likely possess druggable properties that include sufficient water solubility, a half-life justifying once a day dosing, and the capacity to cross the BBB. Although some sEHs are known to cross the BBB, there has not been a systematic process to optimize CNS exposure of sEHs. Formulating and manufacturing compounds that are tailored toward these properties will be crucial to the success of sEHs for treating CNS disorders.

The neuroprotection of sEH inhibition in neurological disorders has been documented in stroke, as evidenced by reduced infarct size accompanied by dampened inflammatory response likely by decreasing pro-inflammatory cytokine secretion. sEHs may also improve outcomes by modulating CBF, although equally compelling evidence suggest non-CBF mechanisms. Future studies must address deficits in current knowledge on stroke relating to vascular involvement, clinical differences between pharmacological inhibition of sEH and gene deletion, and sex differences that may result in different treatment. While sEH inhibition is thought to operate under similar mechanisms in TBI treatment, many more investigations must be pursued to identify the exact targets of sEH-derived pathology in TBI.

The sEH represents a highly unique and innovative target for potential treatment in PD. Studies have shown that sEH levels in both the brain and gut may be a possible PD pathology indicator well before symptoms arise, highlighting its importance in the early stages of the disease. sEH inhibition has also been associated with decreased loss of dopaminergic neurons in the striatum, illustrating its therapeutic power. Moreover, the use of sEH as a PD biomarker and sEHs as a therapeutic agent will likely translate to clinical applications, which can be employed as a basis for other sEH-based investigations in other CNS disorders.

Extending the role of sEH in other neurological diseases remains in its infancy. In epilepsy, sEHs have been shown to reduce inflammation and attenuate GABA-antagonist-induced excitotoxicity. Additionally, a decline in EETs correlates with AB aggregates, suggesting potent vascular effects of sEHs in combating cognitive decline due to VCI and AD. Finally, sEHs may reduce depression-related inflammation, indicating the participation of sEH in psychiatric disorders. Future directions include ascertaining the therapeutic properties and mechanisms of sEH inhibition on the array of these brain disorders.

Currently, stem cell therapies for CNS disorders such as PD, HD, stroke, TBI, and epilepsy are in clinical trials. Optimizing the delivery of exogenous stem cells and the ability to stimulate endogenous stem cells, and overcoming risk factors including tumorigenesis, are avenues to test the adjunctive therapeutic potential of sEHs. CNS disorders are often characterized by chronic inflammation and neuronal death. sEHs can reduce neuroinflammation by upregulating EETs and other EpFAs and promoting angiogenesis, thus alleviating some symptoms of CNS disorders, but also enhancing stem cell grafts’ functional effects. In combination, sEH inhibition can reduce inflammation while the transplantation of exogenous stem cells can provide a neuroprotective effect. Elucidating the exact mechanisms by which sEH inhibition and stem cell therapy interact represents gaps in our knowledge that when resolved can allow a safe and effective combination therapy of sEH and stem cells.

In conclusion, sEHs offer many attractive therapeutic features that may aid in treating a variety of CNS disorders. This review outlined the most studied concepts, as well as highlighted the novelty of sEH use in specific CNS disorders. It is possible that a majority of sEH action comes from preserving EPA and DHA epoxides more than ARA (EETs) and block formation of linoleate diol (leukotxin). Future basic science directions may uncover innovative mechanisms by which sEHs can exert therapeutic effects, thereby guiding translational efforts towards realizing their full clinical potential for treating brain maladies.

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