

Department of Cardiology¹, Xiang-Ya Hospital of Central South University, Changsha; Xin-Jiang Medical University², Urumqi; Department of Pharmacology³, School of Pharmaceutical Science, Central South University, Changsha, PR China

Soluble epoxide hydrolase: a promising therapeutic target for cardiovascular diseases

GUO-HUA NI^{1,2,*}, JIN-FANG CHEN^{1,*}, XIAO-PING CHEN³, TIAN-LUN YANG¹

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Dr. Tian-Lun Yang, Department of Cardiology, Xiang-Ya Hospital, Central South University, Changsha 410008, PR China

nghtt@163.com

*These authors contributed equally to this work.

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Epoxyeicosatrienoic acids (EETs) are cytochrome P450 (CYP450) products of arachidonic acid and EETs are endogenous lipid mediators synthesized by the vascular endothelium which perform important biological functions, including vasodilation, anti-inflammation, antimigratory, and cellular signaling regulations. However, EETs are rapidly degraded by soluble epoxide hydrolase (sEH) to the corresponding diols: dihydroxyeicosatrienoic acids (DHETs), which have little active in causing vasorelaxation. A number of studies have supported that the inhibition of sEH (sEHIs) had cardiovascular protective effects in hypertension, cardiac hypertrophy, atherosclerosis, ischemia-reperfusion injury, and ischemic stroke. Moreover, sEHIs could slow the progression of inflammation, protect end-organ damage and prevent ischemic events, also, attenuate endothelial dysfunction, suggesting that the pharmacological blockade of sEH might provide a broad and novel avenue for the treatment of many cardiovascular diseases.

1. Introduction

Arachidonic acid (AA) is an essential polyunsaturated fatty acid derived from membrane phospholipids, which has potent physiological effects and important cellular functions. AA can be metabolized through three major enzymatic pathways, namely, cyclooxygenases (COX), lipoxygenases (LOX) and CYP450 monooxygenases. The potent biological products of AA metabolism include prostaglandins (PGs), epoprostenol (PGI₂), thromboxane (TXA), as well as leukotrienes and cytokines (Imig and Hammock 2009; Dwyer et al. 2004), all of them termed eicosanoids or eicosanoids.

One critical pathway of the AA cascade is mediated by CYP450 enzymes, which catalysed by hydroxylases and epoxygenases into the endogenous products: 20-hydroxyeicosatetraenoic acid (20-HETE) and EETs, respectively. 20-HETE is a predominant prohypertensive metabolite. EETs have been recognized as endogenous lipid mediators with biological activity and exerted several cardiovascular effects, including dilating coronary arteries by hyperpolarizing of vascular smooth muscle cells (VSMCs), exhibiting anti-inflammatory benefits in endothelial cells (ECs).

EETs have four regioisomeric metabolites: 5,6- EETs, 8,9- EETs, 11,12- EETs and 14,15- EETs, all of them function as autocrine and paracrine mediators on neighboring cells, and exert their effects by released into the extracellular fluid in the vasculature and kidneys (Inceoglu et al. 2008). Additionally, among EETs, 14,15- EETs shows the strongest vasodilator activity, and are the best substrates for sEH in the cardiovascular system. EETs are instable and further taken up by vascular cells and are rapidly converted into the corresponding diols: DHETs and dihydroxyoctadecenoic acids (DiHOMEs) by soluble

epoxide hydrolase (sEH, *EPHX2*) (Minuz et al. 2008). DHETs are thought to be less active and might decrease EETs levels.

2. Biological functions of EETs

EETs are important regulators of vascular homeostasis and inflammation, they exhibit a variety of vascular protective properties, including vasodilation, anti-inflammation, anti-apoptosis, prevention of ischemia stroke, and modulation of several cell signaling cascades (Zhang et al. 2009 b).

2.1. Vasodilation effects

EETs are potent vasodilators which dilate coronary, renal, cerebral arteries and intestinal, whereas they cause vasoconstriction in the lung (Keserü et al. 2008). EETs are widely assumed to be function as endothelium-derived hyperpolarizing factor (EDHF) to induce vessel dilation (Fleming 2008; Fleming and Busse 2006). They are proposed to be as powerful as NO and PGI₂ in modulation of vascular tone and systemic blood pressure, especially in small resistant vessels when NO-mediated vasodilation is impaired (Brähler et al. 2009; Larsen et al. 2008).

EETs exert vasodilation effects depends on hyperpolarization and relaxation of VMSCs. EETs activated large small conductance calcium-activated potassium channels (BK_{Ca}) and intermediate conductance calcium-activated potassium channels (IK_{Ca}) in the VSMCs, which can increase intracellular Ca²⁺ concentration, resulting in K⁺ efflux from the VSMCs, consequently causing hyperpolarization of the endothelial membrane and leading to vasodilation in a number of vascular beds (Behm et al. 2009; Ai et al. 2010).

2.2. Anti-inflammation activity

In recent years, evidence has accumulated that EETs also play a critical role in the processing of anti-inflammation (Revermann et al. 2010a; Schmelzer et al. 2005; Tsai et al. 2010; Zhang et al. 2009a). EETs exert the vascular anti-inflammation and anti-apoptotic effects in ECs by interfering with the activation of nuclear factor κ B (NF- κ B)-mediated gene expression and transcription (Spiecker et al. 2006; Node et al. 1999; Inceoglu et al. 2008). Studies have shown that 11,12-EETs, but not other regioisomeric EETs, or overexpression of CYP2J2, prevented cytokine-induced inflammatory responses and the TNF-induced activation of NF- κ B in ECs, also increased the expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-selectin, decreased leukocyte adhesion to ECs which are involved in the inflammatory response (Li et al. 2009; Deng et al. 2010; Spiecker et al. 2005).

It has also been found that CYP450 enzymes are involved in the activation of nuclear receptor peroxisome proliferator-activated receptor- γ (PPAR γ) by laminar flow, which is involved in anti-inflammatory effects in the artery wall, moreover, EETs are physiological ligands for PPAR γ (Pokreisz et al. 2006). The anti-inflammatory action was initiated by EETs incorporated into a selective EETs membrane phospholipids receptor, and bind to cytoplasmic fatty acid binding proteins (FABP) and PPAR γ to regulate ion channels and intracellular signal transduction pathways (Wang et al. 2010).

Furthermore, EETs have anti-migratory action in VSMCs (Simpkins et al. 2010). 11,12-EETs, 14,15-EETs and overexpression of CYP2J epoxygenase, or inhibition of sEH could attenuate the migration of aortic smooth muscle cells and reduce smooth muscle cell proliferation and migration (Zordoky et al. 2010). The effects of EETs on the anti-migratory and proliferation were produced by EETs activated the cAMP-dependent process that activated BK_{Ca} channels and decreased cyclin D levels in VSMCs, which subsequently affected ECs proliferation and angiogenesis.

3. Soluble epoxide hydrolase and polymorphisms of *EPHX2*

3.1. Soluble epoxide hydrolase

Epoxide hydrolase (EH) comprises a family of enzymes with high abundance in many tissues. The EH family plays important roles in detoxification and conversion of lipid signaling molecules (Marowsky et al. 2009). Two major EHs isoforms in the α/β hydrolase family exist in mammalian cells: the microsomal epoxide hydrolase (mEH, *EPHX1*) and soluble EH (sEH). The mEH is encoded by *EPHX1* gene localized to chromosome q42.1, mEH may detoxify a large variety of xenobiotic-derived epoxides which are involved in the metabolism of environmental contaminants (Nebert and Dalton 2006).

Human sEH is encoded by *EPHX2* and composed of two 62-kDa monomeric subunits, which located to chromosome 8p21-p12 with 555 amino acids. sEH is highly expressed in a number of organs, such as liver, kidney, heart, vasculature and lung, with the liver and kidney showing the highest levels of enzyme activity. The sEH enzyme was a homodimer with each monomer comprised of two distinct structural domains: the carboxy-terminal domain and the amino-terminal domain (Ai et al. 2007). The catalytic residues of sEH are 100% conserved between human and mouse and highly homologous to bacterial haloal-kane dehalogenase (Arand et al. 1996; Cronin et al. 2003). The C-terminal domain is responsible for sEH activity and connected to a smaller N-terminal domain by a proline-rich linker. The N-terminal

domain acts as a phosphatase with specificity for fatty acid phosphates that affects cell signaling, apoptosis and cholesterol synthesis. It has also been reported that the N-terminal domain might promote dimerization of the sEH enzyme, consequently stabilize the EH activity (Newman et al. 2003).

3.2. Association of *EPHX2* genetic polymorphisms with risk of cardiovascular diseases

Accumulating results have suggested that the sequence variation and altered expression levels of *EPHX2* may influence sEH enzymatic activity and metabolism stability, consequently affect the beneficial properties of EETs in the cardiovascular system (Ines et al. 2007; Przybyla-Zawislak et al. 2003; Sandberg et al. 2000; Srivastava et al. 2004). A number of single nucleotide polymorphisms (SNPs) in the human *EPHX2* have recently been implicated to increase susceptibility to the risk of cardiovascular diseases, including increased risk of coronary heart disease (CHD), atherosclerosis, heart failure, hyperlipoproteinemia, type-2 diabetes, and ischemic stroke (Burdon et al. 2008; Fornage et al. 2004; Koerner et al. 2008; Lee et al. 2006; Ohtoshi et al. 2005; Sato et al. 2004; Wei et al. 2007).

Results from the CARDIA study have been shown that the presence of the Arg287Gln polymorphism of *EPHX2* is a significant independent predictor of coronary artery calcification (CAC) in African-American subjects, whereas the association was not observed in white subjects (Fornage et al. 2004). This is also supported by the fact that the Arg287Gln variant allele and different *EPHX2* haplotypes were associated with the increased risk of coronary artery calcified plaque (CorCP) in African American and Caucasians (Burdon et al. 2008). Moreover, the Arg287Gln variant has also been shown to be associated with the carotid artery calcified plaque (CarCP) in the diabetic European American, but not associated with CorCP and intima-media thickness (IMT) in European American and Caucasians (Burdon et al. 2008). Lee et al. (2006) reported that the R287Q variant allele was more common in diabetic CHD Caucasians, but not in African-American individuals, and R287Q has been shown to reduce the EH activity to 25% to 58% *in vivo*, and thus expected to increase EETs levels. It has also been found that smoking further increased the risk associated with Arg287Gln genetic variation (Wei et al. 2007). Another study reported that plasma cholesterol and triglyceride levels were elevated among carriers of the 287Arg allele in familial hypercholesterolemia (Sato et al. 2004).

The ARIC study showed that individuals carrying the K55R polymorphism of *EPHX2* were associated with higher risk of incident CHD, and also demonstrated the higher *in vivo* sEH activity in Caucasians. However, the influence on sEH activity across K55R genotype were not observed in African-Americans (Lee et al. 2006). Similarly, more recent studies reported that the risk of hypertension and the incidence of ischemic strokes were higher in male K55R homozygotes compared with K-carriers, but not in females (Fava et al. 2010). However, another study showed that the K55R polymorphism was not associated with the development of restenosis, or with the increased risk of hypertension in patients over a period of six month after PCI (Kullmann et al. 2009). Another two studies have also indicated that -50T variant allele of CYP2J2 G-50T polymorphism was associated with a significantly lower risk of incident CHD in African-Americans, but not in Caucasians, it also demonstrated the lower CYP2J2 promoter activity and plasma levels of 14,15 DHETs in individuals with the G-50T (Spiecker et al. 2004; Lee et al. 2007).

Three SNPs (SNP 9, SNP 14 and SNP 23) of *EPHX2* showed a significant association with an increased risk of ischemic stroke

in European patients in the ARIC study (Gschwendtner et al. 2008). Interestingly, two common *EPHX2* haplotypes showed apparent and opposing relationships of an association with ischemic stroke risk both in African-Americans and Whites, with one haplotype showed a significantly lower risk of ischemic stroke and another showed higher (Fornage et al. 2005). Taken together, a number of genetic studies have suggested that *EPHX2* could be a potential candidate gene for cardiovascular diseases.

3.3. *sEH* and cardiovascular diseases

Pharmacological utility of exogenous EETs is impractical because they are rapidly degraded into DHETs by sEH, which have been shown to have little active in causing vasoactive properties in the coronary circulation, thus, sEH plays a critical role in the control of EETs levels (Shen et al. 2010; Walkowska et al. 2010). Pharmacological inhibition of sEH has been shown to increase endogenous EETs concentrations (Gross et al. 2008; Revermann 2010b), and so would be expected to enhance EET-mediated vascular protection and act as a promising and an attractive therapeutic agent for the treatment of cardiovascular diseases.

3.4. *sEH* and hypertension

Yu et al. (2000) provided the first evidence that injection a single bolus dose of DCU, a tight binding sEH specific inhibitor, could effectively lower blood pressure, decrease urinary 14,15 DHETs excretion, and increase renal expression of sEH in spontaneously hypertensive rats (SHR). In line with this observation, another two studies have confirmed that administration of sEHI (NCND, AUDA, respectively) successfully prevented further increase in blood pressure in animal models of hypertension (blood pressure was consistently lowered by 25–30 mmHg), and sEHI slowed the progression of renal damage (Imig et al. 2005; Imig et al. 2002). Also, chronic sEHI administration prevented renal vascular and glomerular injury, as well as decreased urinary albumin excretion in rats with Ang II-induced hypertension (Zhao et al. 2004). Two studies have reported that *Ephx2*-deficient mice, which had a markedly lower systolic blood pressure and renal formation of EETs and DHETs, with higher ratios of fatty acid epoxide:diols in plasma, had significantly improved postischemic LVDP recovery and reduced infarct size compared with wild type (WT) animals. Moreover, perfusion with 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE), the putative EETs receptor antagonist, could decrease the cardioprotective phenotype before ischemia (Seubert et al. 2006; Sinal et al. 2000). Recently, it has been reported that there was a significant difference in mean blood pressure between *Ephx2*-null (*Ephx2*^{-/-}) and C57BL/6 mice colony, and each *Ephx2*^{-/-} mouse colony had higher levels of EETs and plasma epoxide:diol ratios (Luria et al. 2007). Study has also shown that mean arterial blood pressure was lower in *Ephx2*^{-/-} DOCA-salt mice compared with wild-type (WT) DOCA-salt mice, furthermore, sEHI (tAUCB) attenuated renal injury and inflammation, and improved glomerular injury in DOCA-salt hypertension (Manhiani et al. 2009).

3.5. *sEH* and ischemia-reperfusion injury

Strong evidences exists for the fact that sEHI had important cardioprotective effects (Chaudhary et al. 2009; Motoki et al. 2008). The hearts of *EPHX2* null mouse had a better recovery of heart function and less infarction area after myocardial ischemia-reperfusion injury *in vivo* (Seubert et al. 2006). Similar effects were seen with a low dose of AUDA which significantly reduced IS/AAR and produced marked cardioprotection in dogs

(Motoki et al. 2008). Furthermore, after injected with AUDA-BE (sEHI) and the EEZE (EET antagonist) to C57B/6J WT or sEH knockout (sEHKO) mice after myocardial I/R, the findings demonstrated that infarction was significantly reduced in sEHKO mice. The results demonstrated that sEHI might provide cardioprotection against myocardial ischemia-reperfusion.

3.6. *sEH* and cardiovascular hypertrophy

There is strong evidence that vascular hypertrophy may benefit from sEHI treatment (Loch et al. 2007; Li et al. 2008). Administration of sEHI (ADU) could attenuate cardiovascular hypertrophy and normalize endothelial function in DOCA-salt-induced rats, although inflammatory cell infiltration and collagen deposition did not change after ADU treatment (Loch et al. 2007). Another study showed that AUDA could inhibit cardiac hypertrophy and remodeling, prevent cardiac arrhythmias associated with cardiac hypertrophy, and increase plasma levels of EETs in stroke-prone spontaneously hypertensive rats (SHRSP), moreover, the effect can be realized by blocking NF-activation in cardiac myocytes (Li et al. 2008).

A recent study demonstrated that a newly developed sEH inhibitor (TUPS) could prevent Ang II-induced cardiac hypertrophy by reducing cardiomyocyte size. Furthermore, consistent with the level and activity of sEH, the expression of hypertrophic markers were elevated significantly in overexpressing sEH cells (Ai et al. 2009). Monti et al. (2008) reported that the spontaneously hypertensive heart failure (SHHF) rat allele was associated with high *EPHX2* expression, moreover, *EPHX2* gene ablation protected against Ang II-induced arrhythmias, the study provided the first evidence that *EPHX2* is a heart failure susceptibility gene.

3.7. *sEH* and ischemic stroke

Recently, the following findings from animal studies have shown that the sEHI may protect against ischemic injury. One study reported that sEHIs (AUDA) protected against cerebral ischemia in spontaneously hypertensive stroke-prone (SHRSP) rats (Simpkins et al. 2009), AUDA effectively reduced percent hemispheric infarct, wall-to-lumen ratio and collagen deposition by attenuating the hypertrophic remodeling of middle cerebral artery (MCA) (Simpkins et al. 2009). Also, AUDA-BE (sEHIs) significantly resulted in at least a 50% reduction in infarct size at 24 h after middle cerebral artery occlusion (MCAO) in mice (Zhang et al. 2007). Another study showed that sEHKO mice sustained a smaller neurological deficit and infarction and higher cerebral blood flow rates following cerebral ischemia after administration of sEHI (AUDA-BE), and the plasma levels of 14,15-DHETs were significantly lower in sEHKO mice, the protection of sEH may contribute to reduce vascular hydrolyase activity and metabolism of circulating 14,15-EETs (Zhang et al. 2008). Corenblum et al. (2008) demonstrated that the sEH enzyme activity in brain and plasma levels of DHETs were significantly lower in stroke-prone (SHR/A3) than in stroke-resistant (SHR/N) SHR. It also showed that animals carriers of SHR/A3 allele of *EPHX2* polymorphism had a greater risk of stroke and end-organ injury (Schmelzer et al. 2005).

4. Conclusion

The fact that both EETs and sEHI have marked cardiovascular protective effects in hypertension, atherosclerosis, cardiac hypertrophy, cardiac and cerebral ischemia, as well as polymorphisms in human *EPHX2*, suggesting that sEHI has a broad pharmacological potential for a wide range treatment of

cardiovascular diseases. As there are functional polymorphisms at the *EPHX2* locus, effects of these polymorphisms on the protective effects of sEHIs are to be studied in future.

References

- Ai D, Fu Y, Guo D, Tanaka H, Wang N, Tang C, Hammock BD, Shyy JY, Zhu Y (2007) Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium *in vitro* And *in vivo*. *Proc Natl Acad Sci USA* 104: 9018–9023.
- Ai D, Pang W, Li N, Xu M, Jones PD, Yang J, Zhang Y, Chiamvimonvat N, Shyy JY, Hammock BD, Zhu Y (2009) Soluble epoxide hydrolase plays an essential role in angiotensin II-induced cardiac hypertrophy. *Proc Natl Acad Sci USA* 106: 564–569.
- Ai D, Shyy JY, Zhu Y (2010) Linking an insect enzyme to hypertension: angiotensin II-epoxide hydrolase interactions. *Kidney Int* 77: 88–92.
- Arand M, Wagner H, Oesch F (1996) Asp333, Asp495, and His523 form the catalytic triad of rat soluble epoxide hydrolase. *J Biol Chem* 271: 4223–4229.
- Behm DJ, Ogbonna A, Wu C, Burns-Kurtis CL, Douglas SA (2009) Epoxyeicosatrienoic acids function as selective, endogenous antagonists of native thromboxane receptors: identification of a novel mechanism of vasodilation. *J Pharmacol Exp Ther* 328: 231–239.
- Brähler S, Kaistha A, Schmidt VJ, Wölffe SE, Busch C, Kaistha BP, Kacik M, Hasenau AL, Grgic I, Si H, Bond CT, Adelman JP, Wulff H, de Wit C, Hoyer J, Köhler R (2009) Genetic Deficit of SK3 and IK1 channels disrupts the endothelium-derived hyperpolarizing factor vasodilator pathway and causes hypertension. *Circulation* 119: 2323–2332.
- Burdon KP, Lehtinen AB, Langefeld CD, Carr JJ, Rich SS, Freedman BI, Herrington D, Bowden DW (2008) Genetic analysis of the soluble epoxide hydrolase gene, *EPHX2*, in subclinical cardiovascular disease in the Diabetes Heart Study. *Diab Vasc Dis Res* 5: 128–134.
- Chaudhary KR, Batchul SN, Das D, Suresh MR, Falck JR, Graves JP, Zeldin DC, Seubert JM (2009) Role of B-type natriuretic peptide in epoxyeicosatrienoic acid-mediated improved post-ischaemic recovery of heart contractile function. *Circ Res* 83: 362–370.
- Corenblum MJ, Wise VE, Georgi K, Hammock BD, Doris PA, Fornage M (2008) Altered soluble epoxide hydrolase gene expression and function and vascular disease risk in the stroke-prone spontaneously hypertensive rat. *Hypertension* 51: 567–573.
- Cronin A, Mowbray S, Dürk H, Homburg S, Fleming I, Fisslthaler B, Oesch F, Arand M (2003) The N-terminal domain of mammalian soluble epoxide hydrolase is a phosphatase. *Proc Natl Acad Sci U S A* 100: 1552–1557.
- Deng Y, Theken KN, Lee CR (2010) Cytochrome P450 epoxygenases, soluble epoxide hydrolase, and the regulation of cardiovascular inflammation. *J Mol Cell Cardiol* 48: 331–341.
- Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, Lulis AJ, Mehrabian M (2004) Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med* 350: 29–37.
- Fava C, Montagnana M, Danese E, Almgren P, Hedblad B, Engström G, Berglund G, Minuz P, Melander O (2010) Homozygosity for the *EPHX2* K55R polymorphism increases the long-term risk of ischemic stroke in men: a study in Swedes. *Pharmacogenet Genomics* 20: 94–103.
- Fleming I, Busse R (2006) Endothelium-derived epoxyeicosatrienoic acids and vascular function. *Hypertension* 47: 629–633.
- Fleming I (2008) Vascular cytochrome P450 enzymes: physiology and pathophysiology. *Trends Cardiovasc Med* 18: 20–25.
- Fornage M, Boerwinkle E, Doris PA, Jacobs D, Liu K, Wong ND (2004) Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: the coronary artery risk development in young adults (CARDIA) study. *Circulation* 109: 335–339.
- Fornage M, Lee CR, Doris PA, Jacobs D, Liu K, Wong ND (2005) The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. *Hum Mol Genet* 14: 2829–2837.
- Gross GJ, Gauthier KM, Moore J, Falck JR, Hammock BD, Campbell WB, Nithipatikom K (2008) Effects of the selective EET antagonist, 14,15-EEZE, on cardioprotection produced by exogenous or endogenous EETs in the canine heart. *Am J Physiol Heart Circ Physiol* 294: H2838–H2844.
- Gschwendtner A, Ripke S, Freilinger T, Lichtner P, Müller-Myhok B, Wichmann HE, Meitinger T, Dichgans M (2008) Genetic variation in soluble epoxide hydrolase (*EPHX2*) is associated with an increased risk of ischemic stroke in white Europeans. *Stroke* 39: 1593–1596.
- Imig JD, Zhao X, Capdevila JH, Morisseau C, Hammock BD (2002) Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* 39: 690–694.
- Imig JD, Zhao X, Zaharis CZ, Olearczyk JJ, Pollock DM, Newman JW, Kim IH, Watanabe T, Hammock BD (2005) An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* 46: 975–981.
- Imig JD, Hammock BD (2009) Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. *Nat Rev Drug Discov* 8: 794–805.
- Inceoglu B, Jinks SL, Ulu A, Hegedus CM, Georgi K, Schmelzer KR, Wagner K, Jones PD, Morisseau C, Hammock BD (2008) Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways. *Proc Natl Acad Sci U S A* 105: 18901–18906.
- Keserü B, Barbosa-Sicard E, Popp R, Fisslthaler B, Dietrich A, Gudermann T, Hammock BD, Falck JR, Weissmann N, Busse R, Fleming I (2008) Epoxyeicosatrienoic acids and the soluble epoxide hydrolase are determinants of pulmonary artery pressure and the acute hypoxic pulmonary vasoconstrictor response. *FASEB J* 22: 4306–4315.
- Koerner IP, Jacks R, DeBarber AE, Koop D, Mao P, Grant DF, Alkayed NJ (2008) Polymorphisms in the Human Soluble Epoxide Hydrolase Gene *EPHX2* Linked to Neuronal Survival after Ischemic Injury. *J Neurosci* 27: 4642–4649.
- Kullmann S, Binner P, Rackebandt K, Hüge A, Haltern G, Lankisch M, Füh R, von Hohenberg E, Bestehorn HP, Scheffold T (2009) Variation in the human soluble epoxide hydrolase gene and risk of restenosis after percutaneous coronary intervention. *BMC Cardiovasc Disord* 9: 48–55.
- Larsen BT, Gutterman DD, Sato A, Toyama K, Campbell WB, Zeldin DC, Manthathi VL, Falck JR, Miura H (2008) Hydrogen peroxide inhibits cytochrome p450 epoxygenases: interaction between two endothelium-derived hyperpolarizing factors. *Circ Res* 102: 59–67.
- Lee CR, North K, Bray MS, Fornage M, Seubert JM, Newman JW, Hammock BD, Couper DJ, Heiss G, Zeldin DC (2006) Genetic variation in soluble epoxide hydrolase (*EPHX2*) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Hum Mol Genet* 15: 1640–1649.
- Lee CR, North KE, Bray MS, Couper DJ, Heiss G, Zeldin DC (2007) CYP2J2 and CYP2C8 polymorphisms and coronary heart disease risk: The Atherosclerosis Risk in Communities (ARIC) study. *Pharmacogenet Genomics* 17: 349–358.
- Li J, Carroll MA, Chander PN, Falck JR, Sangras B, Stier CT (2008) Soluble epoxide hydrolase inhibitor, AUDA, prevents early salt-sensitive hypertension. *Front Biosci* 13: 3480–3487.
- Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, Lu L, Yang J, Mochida H, Low R, Hammock BD, Chiamvimonvat N (2009) Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol* 47: 835–845.
- Loch D, Hoey A, Morisseau C, Hammock BO, Brown L (2007) Prevention of hypertension in DOCA-salt rats by an inhibitor of soluble epoxide hydrolase. *Cell Biochem Biophys* 47: 87–98.
- Luria A, Weldon SM, Kabcenell AK, Ingraham RH, Matera D, Jiang H, Gill R, Morisseau C, Newman JW, Hammock BD (2007) Compensatory mechanism for homeostatic blood pressure regulation in *Ephx2* Gene-disrupted Mice. *J Biol Chem* 282: 2891–2898.
- Manhiani M, Quigley JF, Knightet SF, Tasoobshirazi S, Moore T, Brands MW, Hammock BD, Imig JD (2009) Soluble epoxide hydrolase gene deletion attenuates renal injury and inflammation with DOCA-salt hypertension. *Am J Physiol Renal Physiol* 297: F740–F748.
- Marowsky A, Burgener J, Falck JR, Fritschy JM, Arand M (2009) Distribution of soluble and microsomal epoxide hydrolase in the mouse brain and its contribution to cerebral epoxyeicosatrienoic acid metabolism. *Neuroscience* 163: 646–661.
- Minuz P, Jiang H, Fava C, Turolo L, Tacconelli S, Ricci M, Patrignani P, Morganti A, Lechi A, McGiff JC (2008) Altered release of cytochrome P450 metabolites of arachidonic acid in renovascular disease. *Hypertension* 51: 1379–1385.
- Monti J, Fischer J, Paskas S, Heinig M, Schulz H, Gösele C, Heuser A, Fischer R, Schmidt C, Schirdewan A, Gross V, Hummel O, Maatz H, Patone G, Saar K, Vingron M, Weldon SM, Lindpaintner K, Hammock BD, Rohde K, Dietz R, Cook SA, Schunck WH, Luft FC, Hubner N (2008) Soluble epoxide hydrolase is a susceptibility factor for heart failure in a rat model of human disease. *Nat Genet* 40: 529–537.
- Motoki A, Merkel MJ, Packwood WH, Cao Z, Liu L, Iliff J, Alkayed NJ, Van Winkle DM (2008) Soluble epoxide hydrolase inhibition and gene deletion are protective against myocardial ischemia-reperfusion injury *in vivo*. *Am J Physiol Heart Circ Physiol* 295: H2128–H2134.
- Nebert DW, Dalton TP (2006) The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer* 6: 947–960.

- Newman JW, Morisseau C, Harris TR, Hammock BD (2003) The soluble epoxide hydrolase encoded by *EPXH2* is a bifunctional enzyme with novel lipid phosphate phosphatase activity. *Proc Natl Acad Sci U S A* 100:1558–1563.
- Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, Zeldin DC, Liao JK (1999) Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* 285: 1276–1279.
- Ohtoshi K, Kaneto H, Node K, Nakamura Y, Shiraiwa T, Matsuhisa M, Yamasaki Y (2005) Association of soluble epoxide hydrolase gene polymorphism with insulin resistance in type 2 diabetic patients. *Biochem Biophys Res Commun* 331: 347–350.
- Pokreisz P, Fleming I, Kiss L, Barbosa-Sicard E, Fisslthaler B, Falck JR, Hammock BD, Kim IH, Szelid Z, Vermeersch P, Gillijns H, Pellens M, Grimminger F, van Zonneveld AJ, Collen D, Busse R, Janssens S (2006) Cytochrome P450 epoxygenase gene function in hypoxic pulmonary vasoconstriction and pulmonary vascular remodeling. *Hypertension* 47: 762–770.
- Przybyla-Zawislak BD, Srivastava PK, Vazquez-Matias J, Mohrenweiser HW, Maxwell JE, Hammock BD, Bradbury JA, Enayetallah AE, Zeldin DC, Grant DF (2003) Polymorphisms in human soluble epoxide hydrolase. *Mol Pharmacol* 64: 482–490.
- Revermann M, Schloss M, Barbosa-Sicard E, Mieth A, Liebner S, Morisseau C, Geisslinger G, Schermuly RT, Fleming I, Hammock BD, Brandes RP (2010) Soluble epoxide hydrolase deficiency attenuates neointima formation in the femoral cuff model of hyperlipidemic mice. *Arterioscler Thromb Vasc Biol* 30: 909–914.
- Revermann M (2010) Pharmacological inhibition of the soluble epoxide hydrolase—from mouse to man. *Curr Opin Pharmacol* 10: 173–178.
- Sandberg M, Hassett C, Adman ET, Meijer J, Omiecinski CJ (2000) Identification and functional characterization of human soluble epoxide hydrolase genetic polymorphisms. *J Biol Chem* 275: 28873–28881.
- Sato K, Emi M, Ezura Y, Fujita Y, Takada D, Ishigami T, Umemura S, Xin Y, Wu LL, Larrinaga-Shum S, Stephenson SH, Hunt SC, Hopkins PN (2004) Soluble epoxide hydrolase variant (Glu287Arg) modifies plasma total cholesterol and triglyceride phenotype in familial hypercholesterolemia: intrafamilial association study in an eight-generation hyperlipidemic kindred. *J Hum Genet* 49: 29–34.
- Schmelzer KR, Kubala L, Newman JW, Kim IH, Eiserich JP, Hammock BD (2005) Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc Natl Acad Sci U S A* 102: 9772–9777.
- Seubert JM, Sinal CJ, Graves J, DeGraff LM, Bradbury JA, Lee CR, Goralski K, Carey MA, Luria A, Newman JW, Hammock BD, Falck JR, Roberts H, Rockman HA, Murphy E, Zeldin DC (2006) Role of soluble epoxide hydrolase in posts ischemic recovery of heart contractile function. *Circ Res* 99: 442–450.
- Shen HC (2010) Soluble epoxide hydrolase inhibitors: a patent review. *Expert Opin Ther Pat* 20: 941–956.
- Simpkins AN, Rudic RD, Schreihöfer DA, Roy S, Manhiani M, Tsai HJ, Hammock BD, Imig JD (2009) Soluble epoxide inhibition is protective against cerebral ischemia via vascular and neural protection. *Am J Pathol* 174: 2086–2095.
- Simpkins AN, Rudic RD, Roy S, sai HJ, Hammock BD, Imig JD (2010) Soluble epoxide hydrolase inhibition modulates vascular remodeling. *Am J Physiol Heart Circ Physiol* 298: H795–H806.
- Sinal CJ, Miyata M, Tohkin M, Nagata K, Bend JR, Gonzalez FJ (2000) Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J Biol Chem* 275: 40504–40510.
- Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, Node K, Börgel J, Mügge A, Lindpaintner K, Huesing A, Maisch B, Zeldin DC, Liao JK (2004) Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. *Circulation* 110: 2132–2136.
- Spiecker M, Liao JK (2005) Vascular protective effects of cytochrome p450 epoxygenase-derived eicosanoids. *Arch Biochem Biophys* 433: 413–420.
- Spiecker M, Liao JK (2006) Cytochrome P450 Epoxygenase CYP2J2 and the Risk of Coronary Artery Disease. *Trends Cardiovasc Med* 16: 204–208.
- Srivastava PK, Sharma VK, Kalonia DS, Grant DF (2004) Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure. *Arch Biochem Biophys* 427: 164–169.
- Tsai HJ, Hwang SH, Morisseau C, Yang J, Jones PD, Kasagami T, Kim IH, Hammock BD (2010) Pharmacokinetic screening of soluble epoxide hydrolase inhibitors in dogs. *Eur J Pharm Sci* 40: 222–238.
- Walkowska A, Skaroupková P, Husková Z, Vanourková Z, Chábová VC, Tesar V, Kramer HJ, Falck JR, Imig JD, Kompanowska-Jezierska E, Sadowski J, Cervenka L (2010) Intrarenal cytochrome P-450 metabolites of arachidonic acid in the regulation of the nonclipped kidney function in two-kidney, one-clip Goldblatt hypertensive rats. *J Hypertens* 28: 582–593.
- Wang YJ, Ulu A, Zhang LN, Hammock B (2010) Soluble epoxide hydrolase in atherosclerosis. *Curr Atheroscler Rep* 12: 174–183.
- Wei Q, Doris PA, Pollizotto MV, Boerwinkle E, Jacobs DR Jr, Siscovick DS, Fornage M (2007) Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking. *Atherosclerosis* 190: 26–34.
- Yu Z, Xu F, Huse LM, Morisseau C, Draper AJ, Newman JW, Parker C, Graham L, Engler MM, Hammock BD, Zeldin DC, Kroetz DL (2000) Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ Res* 87: 992–998.
- Zhang LN, Vincelette J, Cheng Y, Mehra U, Chen D, Anandan SK, Gless R, Webb HK, Wang YX (2009) Inhibition of soluble epoxide hydrolase attenuated atherosclerosis, abdominal aortic aneurysm formation, and dyslipidemia. *Arterioscler Thromb Vasc Biol* 29: 1265–1270.
- Zhang W, Koerner IP, Noppens R, Grafe M, Tsai HJ, Morisseau C, Luria A, Hammock BD, Falck JR, Alkayed NJ (2007) Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J Cerebr Blood F Met* 27: 1931–1940.
- Zhang W, Otsuka T, Sugo N, Ardeshiri A, Alhadid YK, Iliff JJ, DeBarber AE, Koop DR, Alkayed NJ (2008) Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. *Stroke* 39: 2073–2078.
- Zhang W, Iliff JJ, Campbell CJ, Wang RK, Hurn PD, Alkayed NJ (2009) Role of soluble epoxide hydrolase in the sex-specific vascular response to cerebral ischemia. *J Cerebr Blood Flow Metab* 29: 1475–1481.
- Zhao X, Yamamoto T, Newman JW, Kim IH, Watanabe T, Hammock BD, Stewart J, Pollock JS, Pollock DM, Imig JD (2004) Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J Am Soc Nephrol* 15: 1244–1253.
- Zordoky BN, El-Kadi AO (2010) Effect of cytochrome P450 polymorphism on arachidonic acid metabolism and their impact on cardiovascular diseases. *Pharmacol Ther* 125: 446–463.