REVIEW

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Soluble epoxide hydrolase: a promising therapeutic target for cardiovascular diseases

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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 biologically products of AA metabolism include prostaglandins (PGs), epoprostenol (PGI_2), thromboxane (TXA), as well as leukotrienes and cytokines (Imig and Hammock 2009; Dwyer et al. 2004), all of them termed icosanoids es 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 (Inceoglua 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 PGI_2 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 Ca^{2+} 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-apoptocic effects in ECs by interfering with the activation of nuclear factor κB (NF-κB)-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-κB 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 (PPARγ) by laminar flow, which is involved in anti-inflammatory effects in the artery wall, moreover, EETs are physiological ligands for PPARγ (Pokreisz et al. 2006). The anti-inflammatory action was initiated by EETs incorporated into a selective EETs membrane phospholipids receptor, and binnd to cytoplasmic fatty acid binding proteins (FABP) and PPARγ 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 have 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 hydrolase 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) SHRs. 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.

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