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Review

Soluble epoxide hydrolase and ischemic cardiomyopathy ☆

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ABSTRACT

Background: The development of cardiovascular disease has been linked to lowered levels of epoxyeicosatrienoic acids (EETs) in the cardiovascular system. Ischemic cardiomyopathy is caused by atherosclerotic lesions in multi-coronary arteries especially diffusive lesions, which can lead to severe myocardial dysfunction, heart enlargement, heart failure, or arrhythmia, and so on. The EETs are metabolized by the soluble epoxide hydrolase (sEH) encoded by the *EPHX2* gene that has several known polymorphisms.
Content: The EPHX2 gene polymorphism is associated with sEH catalytic activity and various cardiovascular diseases. sEH is distributed in a variety of organs and tissues and regulated by multiple factors. Research in the area has led to the presence of multiple powerful soluble epoxide hydrolase inhibitors (sEHIs), whose molecular structure and function has been optimized gradually. sEHIs increase EETs' concentration by inhibiting hydration of EETs into their corresponding vicinal diols. EETs are important signaling molecules and known as endothelium-derived hyperpolarizing factors (EDHF). sEHIs have been developed for their ability to prevent atherosclerosis, dilate the coronary artery, promote angiogenesis, ameliorate postischemic recovery of heart contractile function, decrease ischemia/reperfusion injury, modulate postischemic arrhythmia, and prevent heart failure.
Summary: sEH is one of the etiological factors of cardiovascular diseases, and plays an important role in the progression of myocardium ischemia. This indicates that sEHIs provide a new method for the prevention and treatment of ischemic cardiomyopathy.

sEH was first described in vertebrates in 1972. It is a type of hydrolase that is widely distributed in living beings and belongs to members of the α/ß-fold hydrolase family of enzymes [1]. The epoxide hydrolase family is divided into 5 main subtypes: sEH, microsomal epoxide hydrolase (mEH), leukotriene A4 hydrolase, hepoxilin A3 hydrolase, and cholesterol 5,6-oxide hydrolase [2]. The enzyme was termed cytosolic and later sEH because of its localization largely in the soluble (and peroxisomal) fractions of cell [3,4]. On the subcellular level, it is found in the cytosolic or soluble fraction, but in some cases it can be localized in peroxisomes [5].

1. Introduction of sEH

1.1. Genetic encoding and polymorphisms of sEH

The *EPHX2* gene that encodes sEH is located at chromosomal region 8p21-p12 and contains 19 exons and encodes 555 amino acids. There are 44 known single nucleotide polymorphisms (SNPs) and 1 insertion/deletion of the human *EPHX2* gene. Out of the 44 SNPs identified, 31 occur in introns and 13 occur in exons. Six out of the 13 exonic SNPs result in amino acid substitutions, whereas 7 are silent [6].

Significant research has focused on the genetic polymorphisms of the *EPHX2* gene, their impact on sEH catalytic activity, and their association with various cardiovascular diseases. Eight variable loci were identified in the human *EPHX2* gene, 7 of which were located in the protein coding region, and 2 that resulted in amino acids changes. One study identified 6 SNPs that resulted in amino acid substitutions in the human sEH peptide sequence have been identified [6]. It was shown that the *EPHX2* gene variant is a risk factor for coronary heart disease (CHD): the K55R genetic variation in *EPHX2* was significantly associated with the risk of CHD in Caucasians, implicating EPHX2 as a potential cardiovascular disease-susceptibility gene in the atherosclerosis risk in communities (ARIC) study [7]. In a large multicenter study including 1201 African-American and 1506 non-Hispanic White individuals selected from the participants of the Coronary Artery Risk Development in Young Adults (CARDIA) study, African-American subjects with at least 1 copy of the Gln287 allele had a 2-fold greater risk of coronary artery calcification compared with those not carrying this allele [8]. Consistent with these data, another study conducted in 982 European-American and 176 African- American, subject reported

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that the R287Q polymorphism was associated with coronary artery plaques in European Americans [9].

1.2. Structure and characteristics of sEH

More recently, the sEH gene and transcript have been cloned and the sEH structure and catalytic mechanism were determined. The sEH is a homodimer consisting of 2 monomeric units of 62.5 kDa, whose primary structure suggests that the *EPHX2* gene is produced by the fusion of 2 primordial dehalogenase genes. sEH encoded by *EPHX2* is a bifunctional enzyme [10]. Each monomer contains 2 distinct structural domains connected via aproline-rich linker and both possess catalytic activity. The 35-kDa C-terminal domain is highly homologous to haloalkane dehalogenase which contains a typical α,ß-fold hydrolase structure and EH catalytic activity, and the N-terminal domain is similar to the haloacid dehalogenase and has powerful lipid phosphatase activity as a functional phosphatase and may regulate cholesterol levels *in vivo* and *in vitro*[11]. The N-terminal domain appears to stabilize the epoxide hydrolase activity because expression of the human sEH C-terminal domain alone exhibited reduced activity [12]. Current sEHIs inhibit the epoxide hydrolase activity of the C-terminal domain without affecting the phosphatase activity of the N-terminal domain [13]. The human and mouse sEH amino acid sequences of sEH are 73% homologous [6].

1.3. Expression and regulation of sEH

sEH is now known to be distributed in a variety of organs and tissues and high levels of sEH occur in the intestine, liver, kidney, brain, and vasculature, and lower levels of sEH occur in the lungs, spleen, and testes. Several studies have shown that sEH can be regulated by multiple factors. For example, peroxisome proliferating drugs (agonists of peroxisome proliferator activated receptor alpha) drastically increased cytosolic epoxide hydrolase activity in rodents, and most organisms appeared to respond to these agonists with a 2- to 3-fold increase in hepatic sEH activity. At the same time, treatment for 1 week with a diet containing clofibrate, tiadenol, and acetylsalicylic acid caused an 8-, 13-, and 5- fold increase, respectively, in cytosolic epoxide hydrolase activity in the liver [14]. Diabetes significantly suppressed levels of sEH protein in the liver and kidney. A high concentration of glucose but not ketone bodies suppressed the expression of sEH and the decrease may be due to reactive oxygen species (ROS) generation caused by high glucose [15]. Further study is necessary to elucidate the regulatory mechanism for sEH. In addition, smoking can reduce the activity of sEH and this effect increases with the number of cigarettes smoked per day suggesting that the reduction of activity of the sEH by tobacco smoke decreases the inactivation of carcinogenic epoxides in human lung tissues and therefore may increase a person's susceptibility to lung cancer [16]. Angiotensin II (Ang II), a potent vessel constrictor, elevates blood pressure by acting on several tissue types. Imig [17] et al. showed that kidney sEH protein levels are elevated in AngII hypertension many years earlier. Ai [18] et al. found that Ang II upregulates sEH mRNA and protein in vascular endothelial cells, and that Ang II induced sEH expression in a dose- and time-dependent manner. The authors also found that activator protein 1 (AP-1) is involved in the transcriptional upregulation of sEH by Ang II in vascular endothelial cells *in vitro* and *in vivo*. Furthermore, they pointed out that Ang II can directly upregulate EPHX2 expression. In addition, the level of sEH can be regulated by hormones *in vivo*. sEH activity was measured in the liver and kidneys of male, female, and castrated male mice in order to evaluate sex- and tissue-specific differences in enzyme expression. Greater activity was observed in both the liver and kidneys of adult males than females, and castration of males led to a decrease in activity in both organs but had a greater effect on renal activity than on hepatic activity. These results suggest that testosterone independently regulates sEH activity *in vivo*, and that the kidneys and liver respond differently to testosterone [19] (Fig. 1).

2. sEH inhibition

sEH is an emerging target for pharmacological treatment of cardiovascular diseases because the selective inhibition of sEH leads to increased circulating levels of epoxyeicosatrienoic acids (EETs), resulting in the potentiation of their *in vivo* pharmacological properties. The past decade has seen the rapid development of sEHIs for *in vivo* use and clinical testing. sEH inhibitors were developed as antihypertensive agents, prevent cardiac hypertrophy, decrease vascular smooth muscle proliferation, improve renal hemodynamics, and decreased hypertensive renal damage and so on. sEH inhibitors with improved physical properties and metabolic stability have been developed, and compounds suitable for clinical trials are now available. Mice with lipopolysaccharide(LPS)-induced systemic inflammation, treated with the sEHI, showed reduced production of nitric oxide, cytokines, and pro-inflammatory lipid mediators and significantly improved survival [20]. Another study suggested that sEHI prevented hyperglycemia in diabetic mice [21]. Ulu [22] et al. also found that sEHI could reduce the development of atherosclerosis in apoE-KO mouse model. sEHI significantly improved postischemic left ventricular developed pressure (LVDP) recovery and reduced the infarct size after ischemia and reperfusion, as compared with control hearts in C57BL MI mice [23]. In addition, someone confirmed that sEHI can decrease the incidence of heart failure and arrhythmia in MI mice, and so on.

The first-generation of sEHIs were potent non-competitive inhibitors and included chalcone oxides and glycidol. Unfortunately, these alternative substrates are rapidly inactivated by glutathione and glutathione transferases, making them difficult to use in tissue samples and *in vivo*[13,24]. It was then suggested that urea is the central pharmacophore and that hydrogen bond-stabilized salt bridges were formed between the urea moiety and residues of the C-terminal sEH active site [13]. Amides and carbamates also were found to be potent and stable transition state inhibitors of sEH. Subsequent modifications to improve the *in vivo* stability of sEH

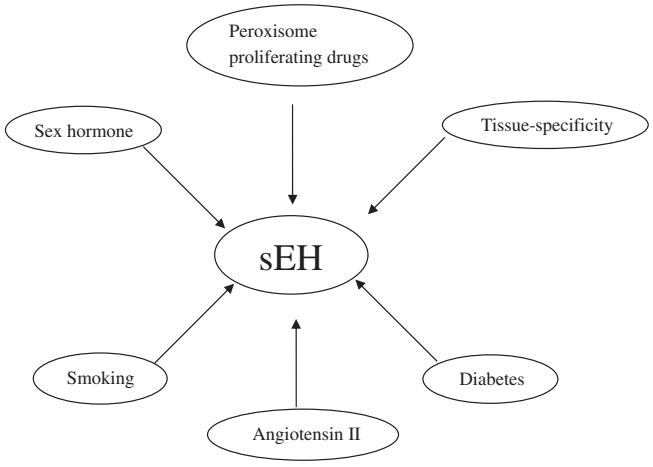

Fig. 1. Soluble epoxide hydrolase(sEH) is distributed very widely. sEH can be regulated by multiple factors *in vivo*. Peroxisome proliferating drugs (agonists of peroxisome proliferator activated receptor alpha) drastically increased cytosolic epoxide hydrolase activity in rodents; diabetes significantly suppressed levels of sEH protein, and the decrease may be due to reactive oxygen species (ROS) generation caused by high glucose; smoking can reduce the activity of sEH; Angiotensin II (Ang II) induced sEH expression in a dose- and time-dependent manner; sEH has sex- and tissue-specific differences in enzyme expression, greater activity was observed in both the liver and kidneys of adult males than females.