

Soluble Epoxide Hydrolase Inhibitors and Heart Failure

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Cardiovascular disease remains one of the leading causes of death in the Western societies. Heart failure (HF) is due primarily to progressive myocardial dysfunction accompanied by myocardial remodeling. Once HF develops, the condition is, in most cases, irreversible and is associated with a very high mortality rate. Soluble epoxide hydrolase (sEH) is an enzyme that catalyzes the hydrolysis of epoxyeicosatrienoic acids (EETs), which are lipid mediators derived from arachidonic acid through the cytochrome P450 epoxygenase pathway. EETs have been shown to have vasodilatory, antiinflammatory, and cardioprotective effects. When EETs are hydrolyzed by sEH to corresponding dihydroxyeicosatrienoic acids, their cardioprotective activities become less pronounced. In line with the recent genetic study that has identified sEH as a susceptibility gene for HF, the sEH enzyme has received considerable attention as an attractive therapeutic target for cardiovascular diseases. Indeed, sEH inhibition has been demonstrated to have antihypertensive and antiinflammatory actions, presumably due to the increased bioavailability of endogenous EETs and other epoxy lipids, and several potent sEH inhibitors have been developed and tested in animal models of cardiovascular disease including hypertension, cardiac hypertrophy, and ischemia/reperfusion injury. sEH inhibitor treatment has been shown to effectively prevent pressure overload- and angiotensin II-induced cardiac hypertrophy and reverse the pre-established cardiac hypertrophy caused by chronic pressure overload. Application of sEH inhibitors in several cardiac ischemia/reperfusion injury models reduced infarct size and prevented the progressive cardiac remodeling. Moreover, the use of sEH inhibitors prevented the development of electrical remodeling and ventricular arrhythmias associated with cardiac hypertrophy and ischemia/reperfusion injury. The data published to date support the notion that sEH inhibitors may represent a promising therapeutic approach for combating detrimental cardiac remodeling and HF.

Introduction

Cardiovascular disease is the leading cause of death in the Western societies [1]. In most instances, heart failure (HF) is the final consequence of a variety of etiologies including coronary heart disease, myocardial infarction, hypertension, arrhythmia, viral myocarditis, and genetic cardiomyopathies. Once heart failure develops, the condition is mostly irreversible. Although considerable progress has been made in the pharmacologic and device management of heart failure in recent decades,

the mortality in HF patients remains significant. Moreover, the incidence and prevalence of cardiac failure are increasing as the population ages [2]. Therefore, novel and effective treatments are desperately needed.

An integral part of the pathogenesis of HF is cardiac remodeling. Cardiac remodeling represents the sum of responses of the heart to a variety of stimuli including ischemia, myocardial infarction, volume, pressure overload, infection, and mechanical injury. These responses, including cardiomyocyte hypertrophy, myocardial fibrosis, inflammation, and neurohormonal activation,

involve numerous cellular and structural changes that ultimately result in a progressive decline in cardiac performance.

There are a multitude of modulating mechanisms and signaling events involved in cardiac remodeling. Arachidonic acid, one of the pivotal signaling molecules previously associated with inflammation, has been implicated as a potential pathway in the pathogenesis of cardiac remodeling [3–4]. Arachidonic acid is released in response to tissue injury and can be metabolized through three major enzymatic pathways. The cyclooxygenase (COX) pathway produces prostanoids. The lipoxygenase (LOX) pathway yields monohydroxys and leukotrienes, while cytochrome P450 (CYP450) epoxygenase pathway generates epoxyeicosanoids. Many of these products are known to be involved in the initiation and propagation of diverse signaling cascades and play central roles in the regulation of myocardial physiology, bioenergetics, contractile function, and signaling pathways.

The CYP450 epoxygenase products, the epoxyeicosanoids, also known as epoxyeicosatrienoic acids (EETs), are major antiinflammatory arachidonic acid metabolites with a variety of biological effects [5]. There is mounting evidence supporting the notion that EETs play a significant protective role in the cardiovascular system. EETs have been identified as potential endothelium-derived hyperpolarizing factors (EDHFs) [6–12]. Major roles of EETs include modulation of both blood pressure and inflammatory signaling cascades. EETs are also associated with a number of other physiological functions including modulation of ion-channel activity, angiogenesis, cell proliferation, vascular smooth muscle cell migration, leukocyte adhesion, platelet aggregation, thrombolysis, and neurohormone release [13–14]. It has been proposed that diminished production or concentration of EETs contributes to cardiovascular disorders [15]. A polymorphism of the human *CYP2J2* gene, which is highly expressed in heart and active in the biosynthesis of EETs, encodes variants with reduced catalytic activity and is independently associated with an increased risk of coronary artery disease [16]. Transgenic mice with cardiomyocyte-specific over-expression of human *CYP2J2* demonstrated enhanced postischemic functional recovery [17] and significant protection against doxorubicin-induced cardiotoxicity [18]. As the protective role of EETs in cardiovascular biology has been increasingly recognized, considerable interest has arisen in developing methods to enhance the bioavailability of these compounds.

There are a variety of pathways involved in the degradation of EETs, but the major pathway is catalyzed by the soluble epoxide hydrolase (sEH) enzyme. sEH converts EETs to their corresponding diols, DHETs, thus modify-

ing the function of these oxylipins [19]. Over the last few years, the sEH enzyme has gained considerable attention as a therapeutic target for cardiovascular diseases [20–23]. Pharmacological inhibition of sEH has emerged as an intriguing approach to enhance the bioavailability of EETs and EET-mediated cardiovascular protective effects [19,24–32]. The beneficial effects of several potent sEH inhibitors in the prevention and reversal of cardiac remodeling due to maladaptive hypertrophy and myocardial ischemia/reperfusion have been demonstrated in several studies, including those from our laboratory [27,30,33–34].

Soluble Epoxide Hydrolase

sEH catalyzes the hydrolysis of the epoxide group of EET regioisomers to form corresponding vicinal diol compounds—the DHETs [19]. sEH was originally found in the liver and kidney and was first assumed to be primarily involved in the metabolism of xenobiotic compounds [35]. It is now known to be distributed in a variety of organs and tissues, where it can modulate the activity of endogenous epoxides, including the EETs [24–25,33,36]. On the subcellular level, it found in the cytosolic or soluble fraction, but in some cases it can be localized in the peroxisomes [37]. Mammalian sEH exists largely as a homodimer of ~62 kDa monomeric subunits [38–39]. Each monomer is comprised of two distinct structural domains, linked by a proline-rich segment [40]. The ~35-kDa C-terminal domain displays epoxide hydrolase activity, while the N-terminal domain exhibits phosphatase activity, which has an undetermined biological role [41]. The homodimeric sEH enzyme possesses a domain swapped architecture, in which the N-terminal domain of one subunit interacts with the C-terminal domain of the other [42]. The structures of murine [43] and human [44] sEH have been solved, providing new insights into the catalytic mechanism, which involves two-step processes. Specifically, the two amino acid residues, asparagine (Asp-333) and tyrosine (Tyr-485), represent the main active sites in the C-terminal domain of both murine and human sEH. The attack on the epoxide group in the substrate by Asp-333 initiates enzymatic activity, leading to the formation of an α -hydroxyacyl-enzyme intermediate. Hydrolysis of the acyl-enzyme occurs by the addition of an activated water, resulting in the regeneration of the active enzyme and the release of the diol product [37,45].

The human sEH is the product of *EPHX2*, a single copy gene on chromosome 8 with 19 exons and 18 introns [38,46]. In rodents, a number of studies have demonstrated pharmacological induction of sEH by

exposure to peroxisome-proliferating activated receptor alpha (PPAR α) ligands like clofibrate, tiadenol, or acetylsalicylic acid [36,47–48], but PPAR α response elements have not been found upstream of human *EPHX2* [49]. Several studies have shown hormonal regulation of sEH in mammals, with sEH activity being elevated in males versus females [48,50–52]. Age-dependent changes in sEH have also been reported in male C57/B6 mice, where sEH activity increased until 15 months after which there was a decline of 59% during senescence [53]. Recently, an API-mediated regulation of sEH by Angiotensin-II has been demonstrated [25], and Monti *et al.* identified a genetic variant in *EPHX2* gene associated with HF in rats, which is characterized by the existence of a new API-binding site in the promoter region [54]. Significant insights into the endogenous role of the sEH have been gained recently. The generation of sEH null mice have provided the first direct evidence for a possible role of sEH in blood pressure regulation [55]. On the other hand, a subsequent study reported that the *Ephx2*-null genotype is not associated with alterations in basal blood pressure. An adaptive response in renal lipid metabolism was observed which may work to maintain normal basal blood pressure in this model [56].

EPHX2 has been identified as a HF susceptibility gene in spontaneously hypertensive heart failure (SHHF) rats [54]. A number of nonsynonymous nucleotide polymorphisms have been identified for human *EPHX2* [57–59] that affect the protein coding sequence as well as enzymatic activity. Of these, sEH variant Lys55Arg, which has increased epoxide hydrolase activity, is associated with coronary artery disease in Caucasians [60]. These studies suggest that sEH may play an important role in the pathogenesis of cardiovascular disease, thus providing rationale for the therapeutic use of sEH inhibitors.

Finally, because polymorphisms in the rat sEH gene have been reported [61], care must be taken when interpreting data from rodent disease models. A recent study examining the susceptibility of different strains of the spontaneously hypertensive rat (SHR) model to the development of brain vascular disease detected multiple variations in the sEH gene [62]. They reported the existence of haplotypes with differing levels of sEH protein and activity. It was further found that brain sEH expression as well as sEH activity was significantly lower in the stroke-resistant rats compared to the stroke-prone rats.

sEH Inhibitors

The first inhibitors discovered for the sEH were epoxide-containing compounds. However, most of these compounds are alternative substrates of the enzyme with

a relatively low turnover that give only a transient inhibition *in vitro* and are inefficient in cell cultures and *in vivo* [35,63–64]. It was not until ureas, amides, and carbamates were found to inhibit the enzyme that compounds could be developed with sufficient *in vivo* stability to unequivocally demonstrate a biological role [65]. Potent compounds in this class are competitive tight-binding inhibitors with pico to nanomolar K_i s that interact stoichiometrically with purified recombinant sEH [65–66] (Table 1). Crystal structures show that the urea inhibitors establish hydrogen bonds and salt bridges between the urea functionality of the inhibitor and residues of the sEH active site, mimicking the intermediate formed during catalysis [43–44,67]. Structural modifications of the 1,3-disubstituted ureas over the last several years have been made to simplify *in vivo* use of these sEH inhibitors. The availability of several X-ray structures has dramatically helped the structure-activity studies aimed toward development of potent inhibitors of the enzyme. Using classical quantitative structure activity relationship (QSAR), 3D-QSAR, and medicinal chemistry approaches, the structure of these inhibitors were improved to yield compounds that have orders of magnitude better inhibition potency than first generation compounds [66,68–70].

The key to developing effective *in vivo* inhibitors is to optimize the absorption, distribution, metabolism, and excretion (ADME) as well as ease of formulation. Earlier compounds such as 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) and its esters and salts have been widely used to evaluate the biological role of the enzyme. However, their poor metabolic stability, relatively high melting point, and limited solubility in water or even many organic solvents make them difficult to use pharmacologically [66,70–71]. AUDA was followed by several compounds which incorporated groups anticipated to bind to hydrogen bonding sites within the catalytic site. One of these compounds, termed 1-adamantan-1-yl-3-(5-(2-(2-ethoxyethoxy)ethoxy)pentyl)urea (AEPU) had dramatically improved water solubility and a lower melting point facilitating formulation [70]. It passed freely through cell membranes and showed efficacy *in vivo* that was better than what would be predicted from blood levels. Newer compounds such as *trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid (*t*-AUCB) and 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea (TPAU) have better oral bioavailability and metabolic stability than their predecessors, possibly due to greater water solubility and resistance to metabolism [72–74]. Following oral administration in the drinking water, a fairly stable blood concentration of *t*-AUCB is maintained for several days [75]. The therapeutic

Table 1 Structures of representative sEH inhibitors

Inhibitor	Structure	IC ₅₀ against human sEH (nM)	References
CDU		85.2	[32,76]
AUDA		3.2	[29,30,77,78]
AUDA-BE		0.8	[27]
AEPU		14.7	[28,33]
t-AUCB		1.3	[34]
TUPS		2.9	[25]
AR9276		1.6	[81]

CDU, 1-cyclohexyl-3-dodecyl urea; AUDA, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid; AUDA-BE, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester; AEPU, 1-adamantany-3-(5-(2-(2-ethoxyethoxy)ethoxy)pentyl)urea; *t*-AUCB, (*trans*-4-(4-(3-adamantan-1-yl-ureido)-cyclohexyloxy)-benzoic acid; TUPS, 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-(trifluoromethoxy-phenyl)-urea; and AR9276, 1-(1-nicotinoylpiperidin-4-yl)-3-(4-(trifluoromethoxy)phenyl)-urea.

effectiveness of these sEH inhibitors has been demonstrated in various animal models of cardiovascular disease [20].

sEH Inhibitors and HF

HF is associated with significant morbidity and mortality attributable largely to progressive myocardial dysfunction accompanied by progressive cardiac remodeling. Persistent pressure/volume overload that occurs in hypertension and cardiac injuries such as myocardial infarction are the most common cause of cardiac remodeling leading to eventual HF. The cardiovascular protective actions of sEH inhibitors were first described in animal models of hypertension [19,32]. There is accumulating evidence to demonstrate that sEH inhibitors lower blood pressure in several animal models of hypertension, such as SHR as well as angiotensin II-induced hypertensive models [19,32,76–78]. Nonetheless, the findings have not been completely uniform. A few studies using different selec-

tive sEH inhibitors failed to show a significant hypotensive effect in SHR [79–80].

Apart from their possible antihypertensive action, sEH inhibitors offer protective effects against cardiovascular disease-related end organ damage [76–77]. Treatment of apolipoprotein E-deficient mice with sEH inhibitors significantly attenuated atherosclerosis development and abdominal aortic aneurysm formation [28,81]. Several studies have documented the cardioprotective roles of sEH inhibition in myocardial ischemia-reperfusion injury [27,30–31]. Mice with targeted disruption of the *EPHX2* gene exhibit improved recovery of left ventricular (LV) developed pressure and less infarction after global ischemia in isolated heart preparation [31] and after regional myocardial ischemia-reperfusion injury *in vivo* [27]. In wild-type mice treated with a sEH inhibitor, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid butyl ester (AUDA-BE), 30 min before ischemia or 10 min before reperfusion, infarct size is significantly reduced [27]. Similar beneficial effects of AUDA were

addressed in a canine model of cardiac ischemia/reperfusion and the combination of the low dose of AUDA with 14,15-EET resulted in a synergistic effect on reducing infarct size (expressed as a percentage of the area at risk) [30].

Using metabolomic profiling and other biological approaches, our group tested the effects of sEH inhibitors on prevention and reversal of cardiac hypertrophy and postischemia remodeling, which are among the most common causes leading to HF. Specifically, we have previously demonstrated that sEH inhibitors can prevent the development of pressure-induced cardiac hypertrophy using a murine model of thoracic aortic constriction (TAC) [33]. In addition, sEH inhibitors reversed the pre-established cardiac hypertrophy caused by chronic pressure overload. Moreover, by using *in vivo* electrophysiologic recordings, our study demonstrated a beneficial effect of the compounds in the prevention of cardiac arrhythmias that are associated with cardiac hypertrophy [33].

Most recently, our laboratory has tested the biological effects of sEH inhibitors on the progression of cardiac remodeling using a clinically relevant murine model of MI [34]. It was demonstrated that sEH inhibitors were highly effective in reducing infarct size and preventing progressive cardiac remodeling post-MI (Figure 1). Moreover, the use of sEH inhibitors resulted in the prevention of electrical remodeling post-MI and in so doing, prevented the propensity for the development of cardiac arrhythmias as assessed by *in vivo* electrophysiologic studies (Figure 2).

A high level of expression of sEH in mouse atrial and ventricular myocytes has been previously documented [33]. To directly test whether MI may up-regulate the sEH expression level, a Western blot was performed using left ventricle free wall tissue from sham, MI and MI animals treated with sEH inhibitors at 3 weeks. Even though no significant differences in the sEH protein expression were observed, the EETs/DHETs ratio was significantly decreased in MI animals, indicating an increased sEH activity in this animal model [34]. Treatment with sEH inhibitors in the MI animals resulted in a significant increase in the plasma ratios of EETs/DHETs compared to MI alone [34].

Possible Mechanisms for the Observed Beneficial Effects of sEH Inhibitors

The underlying mechanisms of the observed beneficial effects of sEH inhibitors in preventing cardiac remodeling due to pressure overload induced hypertrophy and postischemia remodeling have only begun to be addressed. A number of studies have demonstrated that cardiopro-

tection mediated by sEH inhibition is mainly attributed to decreased hydration of EETs to DHETs resulting in higher levels of endogenous EETs. This notion is supported by the observation that pre-reperfusion administration of an EET antagonist, 14,15-epoxyeicosa-5(Z)-enoic acid (14,15-EEZE), abolishes the cardioprotective effect of sEH inhibitors [27,31]. EETs, generated mainly by enzyme CYP2J2 in the heart, have been shown to be cardioprotective [17]. It has been demonstrated that EETs promote postischemic functional recovery in isolated mouse hearts overexpressing *CYP2J2* gene [17,82]. Endothelial *CYP2J2* overexpression also reduces hypoxia-reoxygenation injury [83]. Exogenous administration of 11,12-EET and 14,15-EET produced markedly significant reductions in infarct size in the canine heart, whether given before occlusion or at reperfusion [84].

Using LC-MS/MS based techniques, we have documented a significant decrease in the EETs/DHETs ratio in a MI model, indicating increased sEH activity, which may play a role in the progression of postischemia remodeling [34]. Treatment with sEH inhibitors resulted in the normalization of the ratios of EETs/DHETs and less postischemia LV remodeling (Figure 1A) [34]. sEH enzyme is expressed in the heart and is localized in cardiomyocytes from LV tissue [27]. The expression of sEH is upregulated by angiotensin II in cardiac myocytes *in vitro* and *in vivo*, suggesting a potential regulatory role of sEH in angiotensin II-induced maladaptive hypertrophy [25]. Finally, recent human epidemiological studies have identified associations between variations in EETs metabolic pathway genes and increased cardiovascular risk. A polymorphism leading to reduced gene activity of CYP2J2 is associated with an increased risk of coronary artery disease [16], and *EPHX2* has also been identified as a susceptibility factor for HF [54]. Taken together, these findings suggest that increased sEH activity and reduced bioavailability of EETs may play a significant role in the pathogenesis of HF.

We have previously documented using both *in vivo* and *in vitro* models that sEH inhibitors can block the activation of the nuclear transcription factor (NF- κ B) [33]. NF- κ B is inactive when bound to I κ B, an inhibitory protein that is degraded by proteasomes when phosphorylated by I κ B kinase (IKK) [85–87]. It has been shown that EETs inhibit IKK, preventing degradation of I κ B. This maintains NF- κ B in the inactive state and inhibits NF- κ B-mediated gene transcription [5,88]. Activation of NF- κ B may lead to enhanced oxidative stress, which has been implicated in the various types of HF. Hence, one possible mechanism of the beneficial effect of inhibitors of sEH in heart failure may be a reduction in oxidative stress. In addition, NF- κ B regulates the expression of several genes involved in inflammation, the immune response, apoptosis,

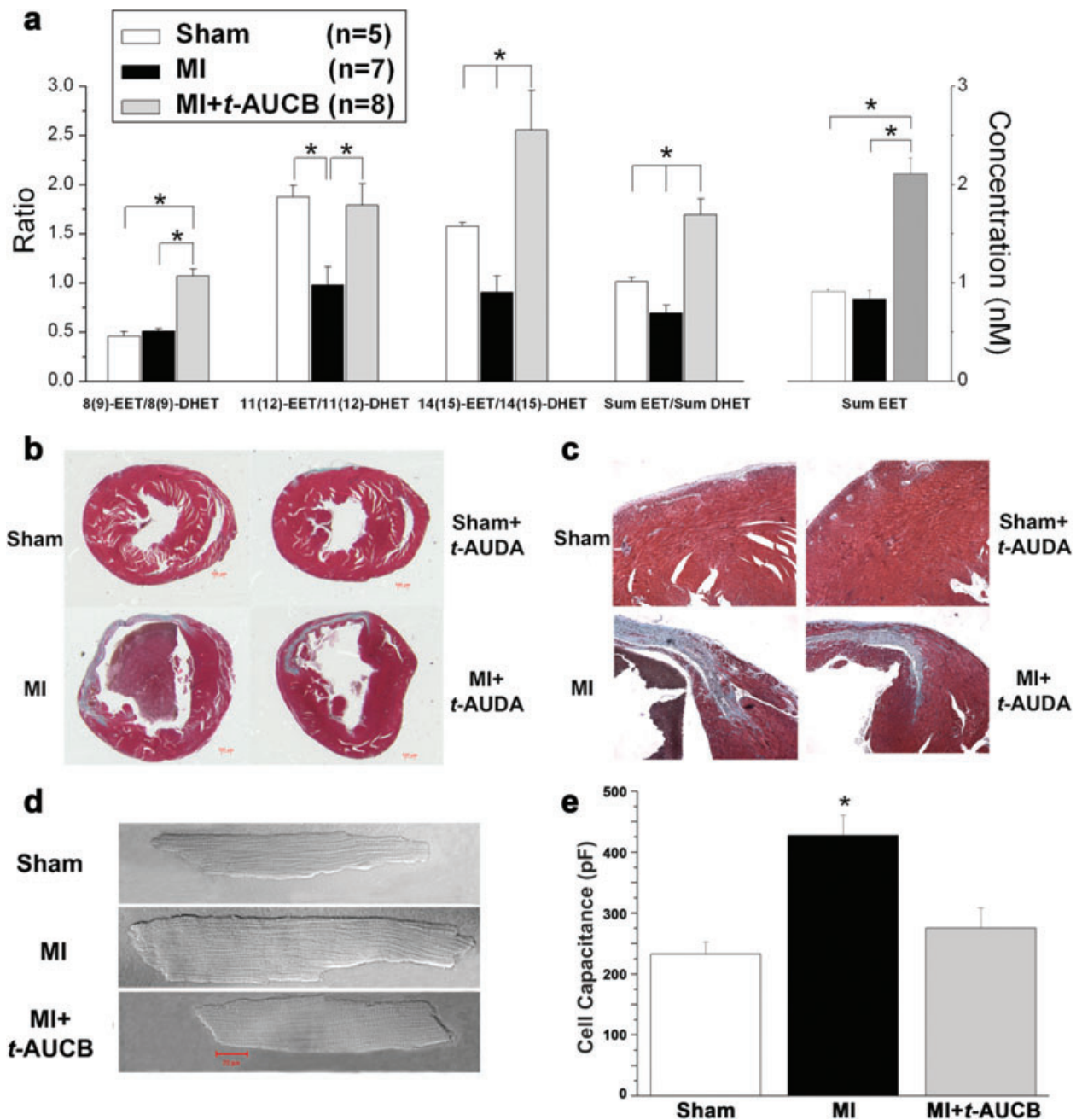


Figure 1 Beneficial effects of sEH inhibitors on LV remodeling in a mouse MI model. **(A)** Plasma level of selected oxylipins from sham operated, MI mice and MI mice treated with *t*-AUCB at 3 weeks of follow up ($*P < 0.05$ comparing sham or treated MI groups to MI alone). These data show that MI in general reduces the fatty acid epoxide to diol ratios while sEH inhibitor administration increases it. **(B)** Histologic sections of sham-operated and MI mouse hearts showing infarct area with scarring and gross cardiac dilatation at 3 weeks in the MI mouse. Treatment of MI mice with *t*-AUCB in drinking water prevented the development of cardiac remodeling. **(C)** Masson's trichrome staining of LV anterior wall, showing connective tissues in

blue. **(D)** Representative photomicrographs of bright-field images of single isolated LV cardiomyocyte remote from infarcted area. **(E)** Summary data of whole-cell capacitance measured from those LV cardiomyocytes ($*P < 0.05$). Treatment with sEH inhibitor reduces the development of myocardial scarring and myocyte hypertrophy. (Adapted from Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, Lu L, Yang J, et al. Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol.* 2009;47:835–845.)

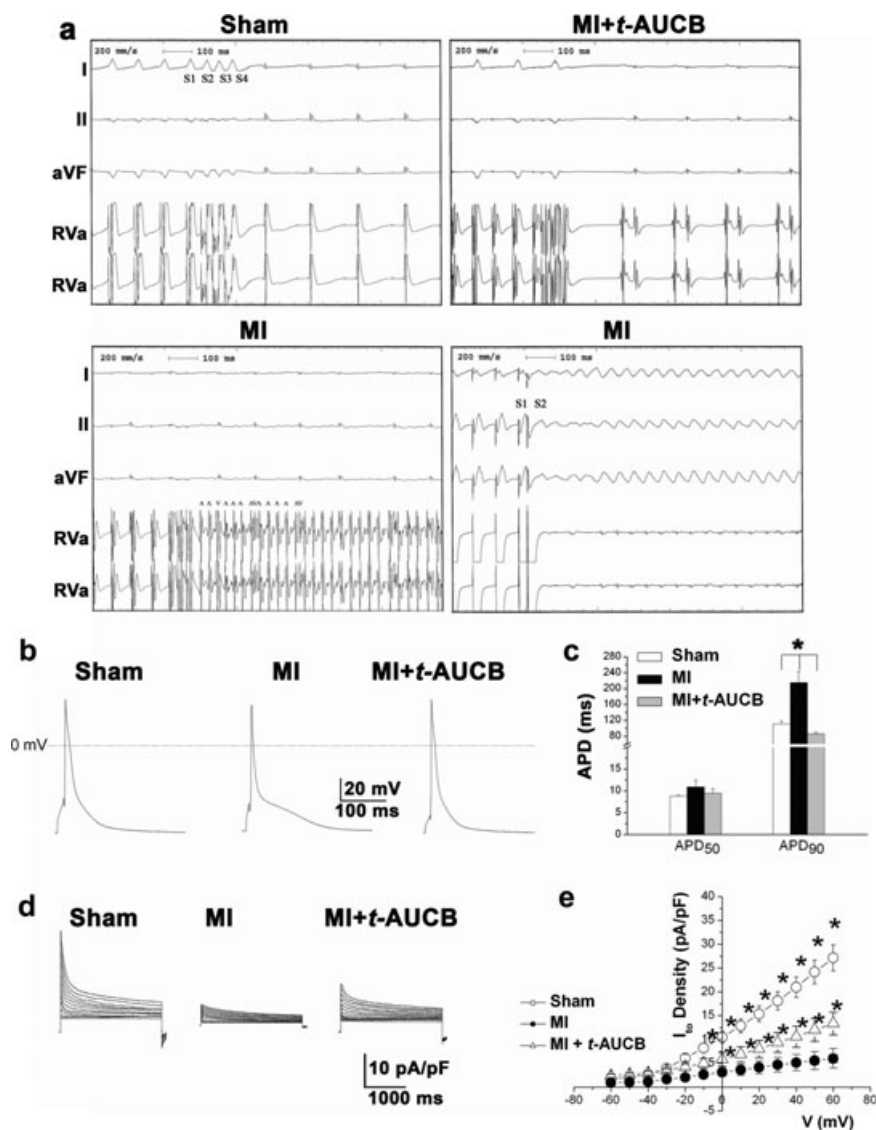


Figure 2 Prevention of cardiac arrhythmia inducibility and electrical remodeling in MI mice by sEH inhibitors. **(A)** *in vivo* ECG in sham and treated compared to untreated MI animals, showing evidence of inducible atrial fibrillation (lower left-hand panel) and ventricular tachycardia (lower right-hand panel), which can be prevented by *t*-AUCB treatment (upper right-hand panel). **(B)** Action potential (AP) recordings from LV free wall myocytes at 3 weeks of follow up. **(C)** Summary data for AP duration at 50 and 90% repolarization (APD₅₀ and APD₉₀ in milliseconds). **(D)** Examples

of Ca²⁺-independent outward K⁺ current traces elicited from a holding potential of -80 mV using test potentials of 2.5 s in duration from -60 to +60 mV in 10-mV increments. **(E)** Summary data for the density of the peak outward components (**P* < 0.05 comparing sham and treated MI animals to MI alone). (Adapted from Li N, Liu JY, Timofeyev V, Qiu H, Hwang SH, Tuteja D, Lu L, Yang J, et al. Beneficial effects of soluble epoxide hydrolase inhibitors in myocardial infarction model: Insight gained using metabolomic approaches. *J Mol Cell Cardiol.* 2009;**47**:835–845.)

cell survival, and proliferation. Many of these same genes are activated during cardiac hypertrophy and LV remodeling [89–93]. Moreover, we have documented that the significant decrease in the EETs/DHETs ratio in the MI model showed a striking parallel with the changes in inflammatory cytokines at 3 weeks post-MI, which indicated a heightened inflammatory state [34]. Additionally, the normalization of the ratios of EET/DHET by sEH

inhibitors results in a reversal of the elevated cytokine levels in the MI model. Persistent inflammation, involving increased levels of inflammatory cytokines, plays a potential pathogenic role in the progression of LV dysfunction and remodeling in HF [94–95]. The sEH inhibitors appear to change the pattern of inflammatory mediators from a state which promotes the propagation of inflammation toward one promoting resolution.

Interestingly, sEH inhibitors have been shown to indirectly downregulate the expression of COX-2 protein and synergize with nonsteroidal antiinflammatory drugs (NSAIDs) toward the reduction of inflammation [96–97]. This suggests that these drug combinations (NSAIDs and sEH inhibitors) can produce a beneficial antiinflammatory effect while reducing the dose needed of COX-2 inhibitors, thus avoiding the adverse cardiovascular side effects attributed to COX-2 inhibitors. The sEH inhibitors also appear to reduce some of the side effects associated with the use of NSAIDs.

EETs are important components of many intracellular signaling pathways as well as modulators for multiple ion channels, therefore the cardioprotective effects of sEH inhibitors may involve modulation of these pathways and ion channel activities. For example, sEH inhibition may activate the PI3K signaling pathway and cardiac ATP sensitive K^+ (K_{ATP}) channels, as suggested in an animal model with targeted disruption of the *EPHX2* gene [31]. A recent study demonstrated that EETs can activate multiple antiapoptotic targets through PI3K/Akt survival signaling and protect cardiomyocytes from hypoxia/anoxia [98]. EETs can increase the opening for both sarcolemmal and mitochondrial K_{ATP} (sarc K_{ATP} and mito K_{ATP}), which are important effectors of protection against cell injury after ischemia and hypertrophy [84,99]. The protective mechanisms contributed by these channels include depolarizing the intra-mitochondrial membrane, altering reactive oxygen species production, and increasing mitochondrial K^+ uptake with attendant reduction of Ca^{2+} overload [100–102].

Moreover, the use of sEH inhibitors can prevent the development of electrical remodeling and ventricular arrhythmias associated with cardiac hypertrophy and myocardial infarction. The susceptibility to increased atrial and ventricular arrhythmias is significantly suppressed in both TAC mice and MI mice treated with sEH inhibitors [33–34]. The cellular electrophysiology in cardiac hypertrophy and failure has been extensively studied in a variety of animal models. The single most consistent abnormality found in these studies is prolongation of action potential duration (APD). The prolongation is due, at least in part, to the reduction in the 4-aminopyridine-sensitive Ca^{2+} -independent transient outward K^+ current (I_{to}) [103]. Indeed, cardiac APDs determine the refractory period of the heart and are precisely and tightly regulated. Excessive prolongation of the APD may predispose cardiac myocytes to early after-depolarizations and life-threatening arrhythmias. Much evidence has shown that various conditions, such as ischemia and HF, where there is increased heterogeneity in cardiac repolarization among different regions of the ventricles, are particularly susceptible to the occurrences of arrhythmias [104].

Treatment with sEH inhibitors prevented the downregulation of I_{to} and APD prolongation which occurs post-MI (Figure 2). In addition, a recent study demonstrated that EETs can enhance the recovery of ventricular repolarization following ischemia by facilitating activation of K^+ channels and PKA-dependent signaling [26].

Future Directions

An increased sEH activity has been demonstrated in an animal model of myocardial infarction, indicating that sEH may play an important role in the progression of postischemia remodeling. However, increased expression level of this enzyme has not been directly detected in the heart. Further studies to explore the mechanism by which sEH activity is dysregulated in MI and possible involvement of other organs such as liver and kidney may help to shed new light on molecular defects in the pathogenesis of myocardial failure. Moreover, in order to definitively determine the best therapeutic utility for sEH inhibitors, future studies to evaluate the potential interactions of sEH inhibitors with other pharmaceuticals seems warranted. It has been shown that regulation of sEH is intimately tied to the renin–angiotensin–aldosterone system (RAAS) in animal models of hypertension and cardiac hypertrophy. sEH inhibitors also synergize with COX-2 inhibitors and other modulators of the arachidonic acid cascade to exert an antiinflammatory effect. Thus, the combination of sEH inhibitors and angiotensin converting enzyme inhibitors or COX inhibitors may provide optimal combination drug therapies with more favorable side effect profiles. Furthermore, to translate the therapeutic utility of sEH inhibitors into clinical intervention in patient care, additional information is needed to identify whether the observed beneficial effects can be generalized to other animal models of HF, such as idiopathic dilated cardiomyopathy, drug-induced heart failure as well as large animal models, since heart failure is a complex clinical syndrome with diverse etiology and a wide array of pathophysiology.

Finally, other cardiolipins besides the ω -6 arachidonic acid metabolites may be relevant to cardiovascular disease. For example, the ω -3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) accumulate in the heart [105] and the epoxides of EPA and DHA are analogs of the EETs. In fact, DHA and EPA epoxides share some of the vasoactive and antiinflammatory properties of the EETs *in vitro* and in some cases have been shown to be more potent [106–107]. DHA and EPA epoxides are, in general, better substrates for sEH than the EETs [Morisseau and Inceoglu, unpublished data], so it is possible that some cardioprotective effects of sEH inhibition

is due to reduction in DHA and EPA epoxide metabolism in the heart.

In summary, research over the past few years with sEH inhibitors in animal models of cardiovascular diseases has suggested that sEH inhibitors have therapeutic potential in a broad range of cardiovascular diseases. Although possible adverse side effects associated with sEH inhibition or genetic deletion have been reported [108–109], the consistent data obtained from several laboratories employing animal models of cardiac hypertrophy and ischemia/reperfusion support the notion that sEH inhibitors may represent a promising therapeutic target for combating detrimental cardiac remodeling and HF.

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Conflict of Interest

NC and BDH have filed patents for the University of California for soluble epoxide hydrolase (sEH) and cardiac hypertrophy therapy.

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