Reno-Protective Mechanisms of Epoxyeicosatrienoic Acids in Cardiovascular Disease

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Abstract:

Cardiovascular disease (CVD) is the leading cause of mortality worldwide and it is well known that end-stage renal disease (ESRD) is a profound consequence of the progression of CVD. Current treatments only slow CVD progression to ESRD and it is imperative that new therapeutic strategies are developed to prevent the incidence of ESRD. Because epoxyeicosatrienoic acids (EETs) have been shown to elicit reno-protective effects in hypertensive animal models, the current review will focus on addressing the reno-protective mechanisms of EETs in CVD. The cytochrome P-450 (CYP) epoxygenase catalyzes the oxidation of arachidonic acid to EETs. EETs have been identified as endothelium-derived hyperpolarizing factors (EDHFs) with vasodilatory, anti-inflammatory, anti-hypertensive and anti-platelet aggregation properties. EETs also have profound effects on vascular migration and proliferation and promote angiogenesis. The progression of CVD has been linked to decreased EETs levels, leading to the concept that EETs should be therapeutically targeted to prevent end-organ damage associated with CVD. However, EETs are quickly degraded by the enzyme soluble epoxide hydrolase (sEH) to their less active diols, dihydroxyeicosatrienoic acids (DHETs). As such, one way to increase EETs level is to inhibit their degradation to DHETs using sEH inhibitors. Inhibition of sEH has been shown to effectively reduce blood pressure and organ damage in experimental models of CVD. Another approach to target EETs is to develop EET analogs with improved solubility and resistance to auto-oxidation and metabolism by sEH. For example, stable ether EET analogs dilate afferent arterioles and lower blood pressure in hypertensive rodent animal models. EET agonists also improve insulin signaling and vascular function in animal models of metabolic syndrome.
Introduction

Cardiovascular disease (CVD) targets the heart or blood vessels inducing a dysfunction of arteries and veins that supply oxygen to vital body organs such as the brain and the heart. In September 2011, the new World Health Organization (WHO) report on CVD prevention and control states that CVD is the leading cause of mortality worldwide. Although a large number of CVD is treatable, the incidence and progression of CVD continues to rise due to lack of adequate preventive measures. An estimated 17.3 million people died from CVD in 2008 and it is anticipated that almost 34 million people will die from CVD by 2030 (WHO report, 2001). In the United States, CVD is the nation's leading killer of both men and women among all racial and ethnic groups. About one million Americans die of CVD annually which represent 42% of all deaths (38, 88). CVD accounts for almost 6 million hospitalizations each year and cause disability for almost 10 million Americans age ≥ 65 years. CVD costs the nation $432 billion each year, including health expenditures and lost productivity (22, 89-90). This cost is estimated to dramatically increase as the burden continues to grow as the population ages. Impaired renal function could occur as a consequence of CVD and often progress to end-stage renal disease (ESRD) (87, 91). ESRD is characterized by extensive albuminuria, increased level of inflammatory cytokines, severe decline in renal function and elevation in blood pressure leading to increased risk of cardiovascular death (87, 91). Many factors contribute to the progression of CVD to ESRD such as environmental pollution, daily stress, lifestyle, tobacco smoking, lack physical activity, obesity, and poor nutrition (87, 120). The pathophysiology of ESRD is multifactorial; however, endothelial dysfunction and vascular inflammation develop with time and are independently associated with mortality (72, 96). Endothelial dysfunction is characterized by impaired vasomotor response, cell proliferation, platelet aggregation, and
vascular permeability which promote vascular inflammation via inducing the production of vasoconstrictor mediators, growth factors and adhesion molecules (72, 96). Although early medical interventions and treatments of CVD are available, lack of treatment options for ESRD are the real challenge in the medical field. Accordingly, new avenues are required to halt the progression of CVD to ESRD.

**Cytochrome P450 metabolites**

Although cyclooxygenase and lipoxygenase metabolites of arachidonic acid are now the most widely accepted clinical targets to treat inflammation and asthma, the cytochrome P-450 (CYP) is a third class of arachidonic acid metabolites that recently emerge as a potential target for treatment of CVD (47). As shown in figure 1, the cytochrome P-450 (CYP) monooxygenase pathway catalyzes the oxidation of arachidonic acid at any of the four double bonds to four regioisomeric epoxideicosatrienoic acids (EETs) (5,6-, 8,9-, 11,12-, and 14,15-EET) by the CYP epoxygenase and/or 19- and 20- hydroxy eicosatetraenoic acids (19- and 20-HETE) by the CYP ω-hydroxylase (34, 47, 95, 106). EETs and 20-HETE have been identified as the main metabolic products of arachidonic acid in rodent and human tissues (47, 117). The members of the CYP2C and CYP4A gene subfamilies are the most functionally relevant renal CYP epoxygenase and CYP ω-hydroxylase, respectively (95). Arachidonic acid metabolites play a crucial role in preserving renal function and regulating blood pressure (95). Initially, pro- and anti-hypertensive effects were proposed for the product of CYP epoxygenase and ω-hydroxylase, respectively (95). Recently, it was shown that 20-HETE has both pro- and anti-hypertensive effects (95). As a pro hypertensive metabolite, 20-HETE is a potent vasoconstrictor, inactivates smooth muscle calcium-sensitive potassium channels and exacerbates the activity of other vasoconstrictor
mediators such as angiotensin II, endothelin and serotonin (95). In contrast, studies in salt-sensitive hypertensive animal models suggest that 20-HETE has antihypertensive and natriuretic properties (117).

EETs are synthesized in the endothelium and are considered important vasodilatory regulators of vascular tone especially when the bioavailability of the endothelium-derived vasodilator nitric oxide is reduced (11, 56, 81). EETs are also endothelium-derived hyperpolarizing factors (EDHFs) in a number of vascular beds, including the renal circulation (10, 57, 109). CYP epoxygenase metabolites and EETs regulate renal sodium and water excretion as indicated by increased CYP epoxygenase activity and urinary EETs excretion following excessive dietary salt intake (129-130). Beside antihypertensive properties, EETs have potent anti-inflammatory properties as previous studies have demonstrated that EETs decrease cytokine-induced endothelial expression of leukocyte adhesion molecules (77). EETs also have thrombolytic activity as they inhibit platelets adhesion to endothelial cells and increase tissue plasminogen activator expression (55, 78). EETs provide protective effects against vascular remodeling via inhibiting the proliferation of vascular smooth muscle cells (108). In endothelial cells, EETs have the opposite effect as they augment cell proliferation and migration suggesting that EETs could improve atherogenesis and promote neovascularization in ischemic diseases (70, 116). EETs also increase endothelial cell survival and attenuate apoptosis (19-20, 68). The versatile effects of EETs make them a therapeutic target for end-organ damage associated with CVD (47) especially after recent reports suggest that genetic variations in the CYP epoxygenase is associated with increased risk of CVD (8, 61). Additionally, the anti-inflammatory and anti-hypertensive properties of EETs also make them a novel strategy to halt the progression of CVD.
to ESRD; however, conversion of EET epoxides to their corresponding diols (DHETs) by soluble epoxide hydrolase (sEH) enzyme limits EETs availability and decreases their beneficial cardiovascular properties.

**Soluble epoxide hydrolase (sEH)**

Microsomal and soluble epoxide hydrolase are two well-known epoxide hydrolase enzymes with different subcellular localization and substrate selectivity (24, 47, 128). The microsomal epoxide hydrolase (mEH) is involved in the metabolism of environmental contaminants whereas soluble epoxide hydrolase (sEH) was initially discovered as a metabolizing enzyme for carcinogenic xenobiotics until it was later found that sEH also metabolizes EETs to less active DHETs (69, 73). The sEH enzyme is found in many tissues such as liver, kidney, lung, heart and ovary and is localized in cytosol, microsomes and peroxisomes (66, 99). The mammalian sEH is a ubiquitously expressed homodimeric enzyme consisting of two 62 kDa monomers (69, 73). Each monomer has two distinct domains, the N-terminal domain with phosphatase activity and the C-terminal domain with sEH activity (26-27, 74). There are no known selective *in vivo* inhibitors of the N-terminal domain and current inhibitors target the C-terminal domain without affecting N-terminal domain (47). Compelling evidence in the literature using wild-type (WT) and sEH gene (*Ephx2*) knock-out hypertensive and diabetic mice models suggest that the phosphatase domain does not contribute to blood pressure regulation or renal injury (23, 67, 101). The human *Ephx2* consists of 19 exons encoding 555 amino acids and has 73 % homology with mouse sEH protein sequences with 100 % conservation in the catalytic residues (85). Clinically, a number of polymorphisms in *Ephx2* that influence sEH enzymatic
activity has been recently reported in CVD suggesting that increased EETs bioavailability could have potential therapeutic benefits in CVD (9, 54, 58, 79, 85).

**EETs signaling mechanisms**

The cellular action of EETs involves either binding to cell surface receptors and/or their intracellular uptake and direct interaction with ion channel or transcription factors. The diverse cell-signaling mechanisms of EETs suggest that EETs may have more than one receptor. Substantial data in the literature demonstrate that EETs bind to selective G-protein coupled receptor to initiate intracellular signaling pathways (47, 56, 105). However, this possibility needs further investigation as long as a putative EET receptor has yet to be clearly identified. EETs-induced vasodilatation and inhibition of the renal sodium-potassium ATPase are potential antihypertensive mechanisms (47, 80). EETs generated by endothelial cells dilate blood vessels via the activation of the GoS protein, adenylate cyclase, to increase cAMP which in turn activates vascular smooth muscle cells large-conductance calcium-activated potassium channels resulting in potassium efflux from the smooth muscle cell and subsequent membrane hyperpolarization (4, 36). There is also evidence for cAMP activation of protein kinase A (PKA) and ADP ribosylation of Gsa cell signaling mechanisms in mediating EETs activation of vascular smooth muscle calcium-activated potassium channels in renal vessels (32, 48, 60). Another alternative mechanism for the EETs vasorelaxant response is the activation of vanilloid (TRP) channel to increase calcium influx leading to endothelial potassium channel activation and hyperpolarization of endothelium which could also trigger vascular smooth muscle relaxation(31, 113). Like endothelial and vascular smooth muscle cells, EETs hyperpolarize platelet cell membranes through activation of calcium-activated potassium channels (55).
EETs exert anti-inflammatory effects via inhibiting the activation of the transcription factor nuclear factor κB (NFκB) (23, 67, 77, 80). For example, over-expression of CYP2J epoxyxygenase in endothelial cells decreased NFκB activation as well as treatment of endothelial cells with 11,12-EET prevented TNF-α-induced NFκB and vascular cell adhesion molecule-1 (VCAM-1) expression (28, 77-78). EETs also mediate the activation of the anti-inflammatory nuclear receptor peroxisome proliferator-activated receptor-gamma (PPARγ) which inhibits NFκB-induced pro-inflammatory adhesion molecules (62).

EETs induce vascular protection via several other autocrine and paracrine effects such as activation of endothelial cells proliferation and migration and inhibition of vascular smooth muscle cells proliferation. EETs-induced endothelial cell proliferation includes the activation of phosphotidyl inositol-3-kinase (PI3K)/AKT pathway, mitogen-activated protein kinase (MAPK) and cAMP/PKA signaling pathways (62, 83-84). The angiogenic effects of EETs in endothelial cells involve the activation of PI3K/AKT, sphingosine kinase-1, and the Proto-oncogene tyrosine-protein kinase SRC-stimulated phosphorylation of STAT-3 (15, 121, 124). The cAMP/PKA cell signaling pathway activation is involved in EET anti-migratory actions in vascular smooth muscle (108). EETs have also shown to inhibit apoptosis via activation of PI3K/AKT pathway to inhibit extracellular signal-regulated kinase (ERK1/2) dephosphorylation (122).

**Enhancement of EETs availability in CVDs**

Recent studies have shown a limited biological activity of EETs in CVD due to their rapid metabolism by sEH to DHETs or by fatty acid β-oxidation to 16-carbon epoxy derivatives (23, 114-115). Although the physiological properties of DHETs are not fully addressed, they
have limited biological activity in comparison to EETs. Elevated EETs levels is now being considered as a new therapeutic target for the treatment of CVD as increased EETs levels protect the overall health of CVD patients from end-organ damage. There are two primary approaches to increase EETs effect \textit{in vivo}. The first approach is to increase EETs survival via the inhibition of EETs degradation by sEH using sEH inhibitors. The second approach is to develop stable EETs agonists that resist auto-oxidation and metabolism by sEH enzyme. Both approaches have recently shown promising evidence in decreasing end-organ damage associated with CVD.

\textbf{Cardiovascular effects of sEH inhibitors}

Inhibition of sEH has been shown to provide protective effects against a variety of CVD such as hypertension, stroke, cardiac hypertrophy, diabetes and atherosclerosis as well as slow the progression of end-organ damage, endothelial dysfunction and inflammation associated with CVD (67, 80, 86, 101, 111, 126).

\textbf{In hypertension}, the vasodilatory effects of sEH inhibitors were tested \textit{in vitro} (51, 57) followed by identifying their antihypertensive effects \textit{in vivo} in experimental models of hypertension such as spontaneously hypertensive rats (SHR), angiotensin hypertension, Ren-2 transgenic rats and deoxycorticosterone acetate (DOCA)-salt hypertension(41, 50-51, 53, 67, 107, 123, 131). Inhibition of sEH also reduced the elevation of blood pressure in high fat diet fed rats and mice and other experimental models of metabolic syndrome (43, 104). Clinically, the sEH inhibitor, AR9281 has recently completed a phase IIA clinical trial in patients with mild to moderate hypertension and impaired glucose tolerance (14).
In heart failure, Ephx2 has been identified as a heart failure susceptible gene in SHR (16, 35). Increased EETs levels, via over-expression of CYP2J, pharmacological inhibition of sEH, or Ephx2 gene deletion, improved post-ischemic left ventricular developed pressure recovery and reduced the infarct size after ischemia/reperfusion injury (5-6, 13). Similar results were obtained in mice and dogs with coronary artery ligation followed by reperfusion as Ephx2 gene deletion or inhibition of sEH also reduced the infarct size (39, 71). Inhibition of sEH provides anti-hypertrophic effects in hypertensive animal models of cardiac hypertrophy such as in DOCA-salt hypertension, stroke-prone SHR and angiotensin II induced hypertension (3, 59, 63) and prevents the development of left ventricular hypertrophy in mice with pressure overload-induced myocardial failure (118). Ephx2 gene deletion also protected myocardium from angiotensin II-induced cardiac arrhythmia and pressure overload-induced heart failure (2-3). However, the clinical use of sEH inhibition or EETs analogs for treatment of heart failure could be limited by the recent observation that Ephx2 gene deletion or sEH inhibition delayed blood pressure recovery and resulted in higher mortality rate in transient cardiac arrest/resuscitation mice model (44).

In stroke, chronic treatment with sEH inhibitor decreased cerebral infarct size after middle cerebral artery occlusion in stroke-prone SHR via improving vascular function and protecting the neurons from cell death independent on blood pressure lowering effects (101-102). Similarly, Ephx2 gene deletion decreased infarct size following a cerebral ischemia (101). Inhibition of sEH also exerted an angiogenic effect in stoke prone SHR as it increased microvascular densities and cerebral artery compliance (21).
**In metabolic syndrome and diabetes**, CYP expression decreased and sEH increased in obese animal models (18, 115) and polymorphism of *Ephx2* gene is associated with insulin resistance in type 2 diabetic patients (79). Studies suggest that EETs is involved in glucose homeostasis and improving insulin signaling during obesity (65, 125). For example, over-expression of CYP2J3 epoxygenase improved insulin resistance in diabetic db/db mice and in fructose-induced insulin resistant rats (119) and inhibition of sEH or *Ephx2* gene deletion decreased glucose levels and improved insulin signal and sensitivity in high fat diet fed mice (65). Inhibition of sEH also decreased blood glucose and improved endothelial function in db/db obese mice (125). In heme oxygenase-2 (HO-2) knock-out mice, another model of obesity with decreased EETs levels, a dual-activity EET agonist/sEH inhibitor improved phenotypic characteristics of metabolic syndrome as it reduced body weight, visceral fat, and blood glucose together with increase EETs levels (104). In type 1 diabetic animal model, *Ephx2* gene deletion or pharmacological inhibition of sEH has recently shown to reduce blood glucose in streptozotocin-induced diabetes (64).

**In atherosclerosis**, polymorphism in *Ephx2* is also linked to incidence of atherosclerosis (58) and inhibition of sEH reduced inflammation and prevented the development of atherosclerotic plaques in apoE-knock-out mice model of atherosclerosis suggesting that increased EETs levels may have therapeutic potential for treatment of hyperlipidemia (111, 126).

**Reno-protective effects of EETs in CVD**

Inhibition of sEH has been shown to protect kidney from end-organ damage in angiotensin II hypertensive rats as it decreased glomerular and tubular collagen expression and vascular hypertrophy and reduced albuminuria (50, 131). Inhibition of sEH also attenuated
afferent arteriolar diameter responses to angiotensin II in isolated kidneys from chronic angiotensin II hypertensive rats (131). Moreover, urinary albumin excretion was decreased and macrophage infiltration was reduced by sEH inhibitor treatment in angiotensin II-salt sensitive hypertension (50). Inhibition of sEH also increased urinary salt and water excretion and decreased renal vascular resistance in angiotensin II infused mice (51). The reno-protective effect of sEH inhibition was quite obvious either during the progression of hypertension or after hypertension was established (49-51, 131). In Goldblatt two-kidney, one-clip (2K1C) hypertensive rats, inhibition of sEH decreased blood pressure and sodium excretion together with improved renal blood flow and glomerular filtration rate (GFR) (107, 114). Inhibition of sEH also provides renal protection in stroke-prone SHR as it decreased blood pressure, renal arteriolar hypertrophy, and proteinuria (59). Ephx2 gene deletion or pharmacological inhibition of sEH also decreased blood pressure and attenuated renal inflammation and glomerular injury in DOCA-salt hypertension (67). Inhibition of sEH could exacerbate the beneficial effects of 20-HETE inhibition as this combination slowed the progression of hypertension and protect kidney from hypertensive induced end-organ damage in Ren-2 transgenic rats (12). In an animal model of metabolic syndrome, increased EETs levels improved abnormal renal hemodynamics and hypertension as inhibition of sEH lowered mean arterial pressure, renal vascular resistance, and GFR and increased renal blood flow in high fat diet fed rats (43). Inhibition of sEH also provides renal protection in angiotensin II induced hypertensive diabetic Goto-Kakizaki rats as it decreased albuminuria and glomerular and tubular injury together with reduced macrophage infiltration and MCP-1 excretion (80). Data from my laboratory demonstrate that renal blood flow was high in Ephx2 knock-out mice compared to control WT. Induction of diabetes with streptozotocin decreased renal blood flow in WT mice and this effect was reduced with either
Ephx2 gene deletion or pharmacological inhibition of sEH (figure 2) suggesting that increased EETs levels via Ephx2 gene deletion or sEH inhibition could prevent the impairment in renal function during diabetes. Ephx2 gene deletion or sEH inhibition also reduced renal injury and inflammation in streptozotocin-induced diabetic mice (23). In addition to their protective effects against chronic progressive kidney disease, sEH inhibitors have shown to provide kidney protection against cisplatin-induced acute renal injury as inhibition of sEH decreased blood urea nitrogen levels and reduced tubular damage induced by cisplatin (82).

Similarly to the effect of sEH inhibition, stable EETs analogs also provide renal protection in CVD. Analogs of 8,9-EET improve glomerular dysfunction as 8-9 EET analogs attenuated focal and segmental glomerulosclerosis permeability factor (FSPF)-induced increased in glomerular albumin permeability(100). 11,12 EETs analogs also mediate afferent arteriolar relaxation utilizing the phospho-protein phosphatase 2A (PP2A) to activate the large-conductance calcium-activated potassium channels (45). Similarly, 11,12-EET agonists exerted vasodilatory and anti-inflammatory effects via the inhibition of TNF-α induced VCAM-1 expression (28). In vivo, the potential protective effects of EETs agonists against end-organ damage in CVD have been recently explored in animal models of hypertension and metabolic syndrome (46, 104). EETs agonists dilated afferent arterioles and lowered blood pressure in SHR (46). A dual-activity EET agonist/sEH inhibitor has recently been tested in HO-2 knock-out mice, a model of metabolic syndrome with decreased EET levels and impaired vascular function and found to improve metabolic syndrome phenotype and endothelial dysfunction (104). Recent data from Dr. John Imig laboratory demonstrated that EET agonist also decreased blood pressure and reduced renal injury and inflammation in angiotensin II-induced hypertensive rats. Overall,
EETs ability to improve renal vascular function and decrease renal injury could provide a promising role of sEH inhibitors in treatment of acute and chronic kidney disease.

**Reno-protective mechanisms of EETs in cardiovascular diseases**

The potential reno-protective mechanisms of EETs are summarized in figure 3 and as follows;

**A- EETs hypotensive and hypoglycemic effects**

EETs improve renal function and protect the kidney from renal inflammation and injury associated with the progression of CVD as they increase renal blood flow, GFR and sodium excretion and decrease renal vascular resistance (47, 105-106). Previous studies suggest that the antihypertensive effect of EETs mediates their reno-protective effects as inhibition of sEH lowered blood pressure together with reduced renal injury in hypertensive and metabolic syndrome animal models (43, 50, 131). However, the antihypertensive effect of sEH inhibitors is not the sole reno-protective mechanism as inhibition of sEH maintained renal protection in hypertensive diabetic Goto-Kakizaki independent on blood pressure lowering effects (80). Moreover, the Ephx2 deletion demonstrated variable results on blood pressure in hypertensive and diabetic mice models (23, 67, 103, 127) though these mice exhibited reduced renal injury when diabetes was experimentally induced despite blood pressure lowering effects (23).

Similarly, Ephx2 deletion or sEH inhibitors have also been shown to reduce blood glucose and enhance glucose utilization via increased insulin release, reduced β-cell apoptosis, and decreased insulin resistance in obese and diabetic mice (64-65). The hypoglycemic effect of sEH inhibition could be also considered a potential reno-protective mechanism against diabetic-induced renal injury; however, Ephx2 gene deletion or pharmacological inhibition of sEH failed to lower blood glucose in steptozotocin-induced diabetic mice though it provided renal
protection against diabetic-induced renal injury (23). Collectively, the hypotensive and hypoglycemic effects of sEH inhibition could not completely explain the reno-protective effects of EETs in CVD and additional reno-protective mechanisms of EETs in CVD are yet to be explored.

**B- EETs anti-inflammatory effects**

It well known that inflammatory cytokines are key components in end-organ damage associated with CVD (7). The relationship between inflammatory cytokines and CYP epoxygenase has been recently investigated. Cytokines decreased CYP epoxygenase expression and its vasodilatory effects whereas blocking inflammatory cytokines effects, such as inhibition of tumor necrosis factor-alpha (TNF-α) or chemokine receptor 2b (CCRb2) blockade, increased renal CYP epoxygenase expression and decreased renal injury and inflammation in angiotensin II-salt-sensitive hypertension (24-25, 33-34). Accordingly, increased EETs level via the inhibition of sEH could provide renal protection against end-organ damage in CVD via EETs anti-inflammatory effects. EETs have been shown to exert anti-inflammatory effects via the inhibition of NFκB inflammatory signaling activation (23, 67, 77, 80). For example, inhibition of sEH reduced the production cytokines and pro-inflammatory mediators in lipopolysaccharide (LPS)-induced inflammation in mice with improved survival rates (98). Inhibition of sEH reduced macrophage infiltration in angiotensin II-salt sensitive hypertensive rats and in hypertensive diabetic Goto-Kakizaki rats (80, 131). Pharmacological inhibition of sEH or Ephx2 gene deletion inhibited renal NFκB activation and decreased MCP-1 excretion in DOCA-salt hypertensive mice (67). We have recently shown that Ephx2 gene deletion also inhibited renal inflammation via inhibition of phospho-IκB kinase (P-IKK)-induced NFκB signaling activation in streptozotocin-induced diabetic mice (23). Inhibition of sEH exerted analgesic effects and
reduced COX2 expression in lipopolysaccharide-induced hyperalgesia (97). Additionally, inhibition of sEH also reduced the number of renal apoptotic/necrotic cell in right kidney from WT mice subjected to 45 minutes ischemia followed by 4 hours perfusion as well as in the left contralateral control (figure 4). Accordingly, the anti-inflammatory and anti-apoptotic effects of EETs could be a potential reno-protective mechanism against CVD-induced renal injury.

**C- EETs-PPAR interaction**

Peroxisome proliferator-activated receptor-alpha (PPAR-α) activator is involved in regulating fatty acid metabolism and attenuates vascular smooth muscle cell proliferation (40, 92). PPAR-α agonists have been shown to reduce renal injury, oxidative stress and inflammation during CVD with or without blood pressure lowering effects (37, 42-43). EETs have been shown to activate PPAR-α (30, 75) and inhibition of sEH also activates PPAR-α and inhibits cyclin D1 (29, 76). Accordingly, the ability of sEH inhibitors to activate PPAR-α could be beneficial in protecting kidney from end-organ damage associated with patients with hyperlipidemia, diabetes and hypertension. EETs also increase PPAR-γ transcription activity where PPAR-γ agonists are insulin sensitizer and are key elements in inhibiting NFκB-induced inflammation (62). Current commercial available PPAR-γ agonists for treatment of diabetes have unwanted fluid retaining effect, which is detrimental to patients with CVD especially those with heart failure. Accordingly, using sEH inhibitor with or without PPAR-γ agonist could provide a new avenue for treatment of cardiometabolic syndrome and protect kidney for ESRD because EETs diuretic and natriuretic effects could lessen the fluid retaining properties of PPAR-γ agonist.

**D- EETs-heme oxygenase relationship**

Heme oxygenase (HO) is the primary pathway for heme metabolism generating biliverdin, iron, and CO and biliverdin which is further metabolized to bilirubin (1, 110). Two
isoenzymes of HO are well known; HO-1 and HO-2. HO-1 is induced in response to many pathophysiological changes in CVD such as hypoxia, oxidative stress, ischemia, and increased cytokines whereas HO-2 is the constitutive isoenzyme that accounts for most HO activity in normal healthy state (1, 110). Induction of HO-1 provides protective effects via the dissipation of the pro-oxidant heme and the generation of the antioxidant, anti-inflammatory and anti-apoptotic metabolites (1, 110). It is well known that HO-1 induction could protect kidney from end-organ damage in CVD via anti-oxidant and inflammatory effects as induction of HO-1 have been shown to inhibit NADPH-derived oxidative stress and inflammation; however, the use of hemeoxygenase-1 inducers are limited due to their potential toxic effects (1, 17, 110). EETs and HO share overlapping anti-inflammatory properties and the possible link between them have been initially established in vitro. Activity and expression HO-1 increased in cultured endothelial cells treated with 11,12-EET. 11,12-EETs vasorelaxant properties in mesenteric vessel was also shown to be mediated by increased HO activity (93-94). EETs agonist or sEH inhibition inhibited adipogenesis and decreased levels of inflammatory cytokines together with increased HO-1 expression in mesenchymal stem cell (MSC)-derived adipocytes proliferation and differentiation and inhibition of HO activity reversed EET-induced inhibition of adipogenesis (52, 112). In vivo, Sodhi et al. demonstrated that a dual-activity EET agonist/sEH inhibitor increased renal EETs levels and renal HO-1 expression, and these changes were associated with a reduction in inflammatory cytokines levels and restoration of aortic rings relaxation to acetylcholine together with improved metabolic syndrome phenotype in obese HO-2 knock-out mice (104). We have recently shown that renal HO-1 expression and activity increased in diabetic Ephx2 knock-out mice with no change in HO-2 expression and these changes were associated with decreased renal oxidative stress, inflammation and damage compared to diabetic
WT mice (23). Furthermore, Inhibition of HO activity negated the reno-protective effects of Ephx2 gene deletion or sEH inhibition during diabetes as it increased inflammatory and renal injury markers (23). Overall, increased EETs levels induce HO-1 in CVD which in turn protects the kidney from end-organ damage via the reduction in oxidative stress and the inhibition of NFκB induced inflammation.

**Perspectives and Significance**

sEH inhibitors or EET agonists have potential therapeutic use to slow the progression of ESRD associated with CVD especially after a sEH inhibitor has recently completed phase IIA clinical trial in hypertensive patients with impaired glucose tolerance (14). Because the inhibition of sEH has been shown to reduce COX2 expression (97), sEH inhibitors could be used in combination with traditional non-steroidal anti-inflammatory drugs or selective COX-2 inhibitors to synergize their anti-inflammatory properties in CVD and reduced their cardiotoxic side effects. Another potential approach to halt the progression of ESRD is to investigate the effect of combined administration of EET agonist and sEH inhibitor on the progression of ESRD in CVD. A dual EET agonist and sEH inhibitor improved endothelial function and metabolic syndrome phenotype and reduced blood pressure in obese HO-2 knock-out mice (104). Gross et al. recently showed that sEH inhibitor or exogenous EET exert cardio-protection in canine ischemia/reperfusion and combined administration of both resulted in a synergistic effect (39). Accordingly, future clinical studies should determine whether combined administration of EET agonist and sEH inhibitors will provide better reno-protective effects than either treatment alone. Moreover, the potential use of 20-HETE inhibitor and sEH inhibitor or EET agonist could also provide favorable synergistic effects against ESRD in CVD especially after recent published data demonstrated that combined inhibition of 20-HETE formation and sEH inhibition attenuated the
development of hypertension and protected the kidney from hypertension-induced injury in Ren-2 transgenic rats (12).

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**Figure Legends**

**Figure (1):** Overview of arachidonic acid metabolisms to eicosanoids. Arachidonic acid is metabolized by cyclooxygenase to prostaglandins and by lipoxygenase to leukotrienes. Prostaglandins and leukotrienes play important role in the progression of inflammation and asthma, respectively. The third pathway of arachidonic acid involves its conversion by cytochrome P-450 (CYP) where CYP ω-hydroxylase catalyzes the formation of 20-HETE and CYP epoxygenase catalyzes the formation of four epoxyeicosatrienoic acid regioisomers (EETs). 20-HETE has both pro- and anti-hypertensive properties; however, EETs are endothelium-derived hyperpolarizing factors with anti-inflammatory and anti-hypertensive properties. The biological activity of EETs is limited by their rapid metabolism by soluble epoxide hydrolase (sEH) to less active dihydroxyeicosatrienoic acids (DHETs).

**Figure (2):** Non invasive ultra-sound imaging technique was used to determine total renal blood flow velocity using pulse-wave doppler in the renal artery after six weeks of induction of diabetes with streptozotocin (65 mg/kg, ip) in wild type (WT), with or without treatment with the sEH inhibitor trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (AUCB, 10 mg/L in drinking water), and Ephx2 mice and % change of renal blood flow was calculated from control WT. Ephx2 gene deleted mice had a higher renal blood flow than control WT which could be attributed to increased EETs levels. Induction of diabetes decreased renal blood flow in diabetic WT and this change was reduced by either Ephx2 gene deletion or pharmacological inhibition of sEH (n=3 per group).

**Figure (3):** Schematic diagram of the possible reno-protective mechanisms of EETs in cardiovascular disease. The hypoglycemic and hypotensive effects of EETs could protect kidney from diabetic and hypertensive induced renal injury. EETs also inhibit vascular smooth muscle...
cell proliferation and exert anti-inflammatory properties via different pathways such as induction of HO-1, increased eNOS, activation of PPAR, inhibition of NFκB, and decreased COX-2 and these mechanisms could provide renal protection against the progression of end stage renal disease.

**Figure (4):** Assessment of renal apoptosis/necrosis by flow cytometry in WT mice with or without AUCB treatment for a week (10 mg/L in drinking water). Right kidney was subject to ischemia for 45 minutes followed by reperfusion for 4 hours. Percentage of renal apoptotic/necrotic cells was elevated in right compared to left kidney and inhibition of sEH with AUCB treatment reduced this percentage in both kidneys (n=3 per group).
References


Cytochrome P450 metabolites

Prostaglandins → Cyclooxygenase

Arachidonic acid

CH₃

CYP450

Hydroxylases

Epoxigenases

20-HETE
Pro-hypertensive
Anti-hypertensive

EETs

- Vasodilation
- Anti-hypertensive
- Anti-inflammatory
- EC proliferation
- Anti-apoptotic
- Platelet aggregation

DHETEs (less active)

Figure 1
Figure 2
Reno-protective mechanisms of EETs in cardiovascular disease

Figure 3
Figure 4