HHS Public Access

Author manuscript

Hypertension. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Hypertension. 2020 July ; 76(1): 3–15. doi:10.1161/HYPERTENSIONAHA.120.13898.

Epoxy Fatty Acids: from Salt Regulation to Kidney and Cardiovascular Therapeutics

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Abstract

Epoxyeicosatrienoic acids (EETs) are epoxy fatty acids that have biological actions that are essential for maintaining water and electrolyte homeostasis. An inability to increase EETs in response to a high salt diet results in salt-sensitive hypertension. Vasodilation, inhibition of epithelial sodium channel, and inhibition of inflammation are the major EET actions that are beneficial to the heart, resistance arteries, and kidneys. Genetic and pharmacological means to elevate EETs demonstrated anti-hypertensive, anti-inflammatory, and organ protective actions. Therapeutic approaches to increase EETs were then developed for cardiovascular diseases. Soluble epoxide hydrolase (sEH) inhibitors were developed and progressed to clinical trials for hypertension, diabetes, and other diseases. EET analogs were another therapeutic approach taken and these drugs are entering the early phases of clinical development. Even with the promise for these therapeutic approaches, there are still several challenges, unexplored areas, and opportunities for epoxy fatty acids.

Keywords

epoxyeicosatrienoic acid; natriuresis; endothelium; inflammation; sodium channels

INTRODUCTION

Kidney and cardiovascular diseases are inextricably linked: the kidney is essential for renin secretion, as well as water and electrolyte homeostasis. Human and animal studies conducted by Dr. Lewis K. Dahl demonstrated that excessive salt intake contributed to hypertension.[1,2] Over a half century since these key findings by Dr. Dahl and other investigators connecting salt, kidney, and hypertension there has been strides in the treatment of cardiovascular and kidney diseases. Salt restriction and diuretics represented successful approaches to treating hypertension and preventing cardiovascular events.[3,4] Nevertheless, the cardiovascular disease hypertension still afflicts one in every four people and remains the leading cause of death worldwide.[5,6] Chronic hypertension is a leading contributor to

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CONFLICT OF INTEREST STATEMENT

Dr. Imig has patents that cover the composition of matter for EET analogs, sEH inhibitors, bifunctional sEH inhibitors. There are no other conflicts of interest, financial or otherwise, are declared by the author.

cardiovascular, renal, and cerebral disease morbidity, and mortality, and prevalence and multiple medical and socio-economic consequences make it a major health challenge. While it is widely accepted that early clinical intervention minimizes the devastating consequences of hypertension, a lack of obvious symptoms complicates its timely diagnosis and the prevalence of uncontrolled hypertension is approximately 50%.[7,8] A significant portion of patients with hypertension develop chronic kidney disease (CKD) and progress to end-stage renal disease.[9,10] Kidney diseases such as CKD afflict one out of every ten Americans and continue to lack proper treatments.[9] Accordingly, there continues to be a significant need for finding new approaches to treat cardiovascular and kidney diseases.

Common dysfunctional mechanisms that contribute to cardiovascular and kidney diseases include water and electrolyte regulation, inflammation, and endothelial function. Humans over recent centuries have adapted to a dramatic increase in sodium intake and a reduction in potassium intake.[11,12] The kidney's relative inability to adapt to this change in dietary salt has led to a substantial increase in salt-sensitive hypertension.[12] Evidence from genetic polymorphisms and monogenic hypertension has led to the postulate that hypertension is the consequence of multiple genetic polymorphisms that in aggregate result in defective kidney sodium excretion.[13,14,15] Direct evidence that impaired kidney function contributes to hypertension has been demonstrated in kidney transplantation studies both in rats and humans.[16,17] Inflammation – in particular kidney inflammation – has been demonstrated to contribute to hypertension and kidney diseases.[18,19,20] Likewise, endothelial dysfunction contributes to salt-sensitive hypertension and is a strong indicator of poor kidney and cardiovascular health.[21,22] These dysfunctional mechanisms are likely interrelated and act in concert to cause and contribute to the progression of cardiovascular and kidney diseases.

Epoxy fatty acids provide a pathway that can improve water and electrolyte regulation, decrease inflammation, and improve endothelial function in salt-sensitive hypertension and CKD.[23,24] Likewise, genetic manipulation decreasing epoxy fatty acids and decreasing kidney epoxy fatty acids have been associated with salt-sensitive hypertension.[24,25] Experimental evidence in animal models and humans revealed that genetic or pharmacological manipulation increasing bioavailability of epoxy fatty acids, epoxyeicosatrienoic acids (EETs), improves cardiovascular and renal function.[25,26,27] The focus of this review is to present how evaluating EET actions in salt regulation evolved into the development of therapeutics that demonstrate great promise for treating kidney and cardiovascular diseases.

EPOXY FATTY ACIDS – BIOLOGICAL ACTIONS

Cytochrome P450 (CYP) epoxygenase enzymes form epoxides by acting on carbon double bonds of fatty acids. Arachidonic acid is the most abundant fatty acid in mammals and is acted upon by CYP epoxygenase enzymes to form four regioisomeric EETs (Table 1).[28] The major CYP epoxygenase enzymes are the CYP2C and CYP2J; CYP2C is the main epoxygenase enzyme responsible for kidney and endothelial EET generation.[29,30] Of the four EET regioisomers, 11,12-EET and 14,15-EET are the major products of the human CYP2C8 and CYP2C9, the mouse Cyp2C44 and the rat CYP2C23.[29,30,31] Following generation, EETs can then be acted upon by soluble epoxide hydrolase (sEH), which adds

water across the epoxide bond forming a diol (DHET). The rank order affinity for EETs for sEH is: 14,15-EET > 11,12-EET > 8,9-EET > 5,6-EET.[23] In general, EETs are biologically active, whereas DHETs are less active or inactive in terms of vascular, renal, and inflammatory actions.[22,23]

The finding that EETs are endothelium-derived hyperpolarizing factors (EDHFs) resulted in extensive evaluation in several organ vasculatures.[32,33] Early studies demonstrated that EETs – in particular 11,12-EET and 14,15-EET – vasodilate renal, coronary, cerebral, and mesenteric arterioles (Figure 1).[23] Renal afferent arterioles dilated to 11,12-EET and 14,15-EET but failed to respond to their corresponding DHETs.[34] Finding that DHETs were inactive provided initial evidence that inhibiting sEH could increase the EET-mediated vasodilation. Although 8,9-EET and 5,6-EET have been demonstrated to be vasodilatory in some vasculatures, it was found that in renal afferent arterioles 8,9-EET was inactive and 5,6-EET caused cyclooxygenase-dependent (COX) constriction of renal afferent arterioles.[34,35,36,37] As for the cellular site of action, dilation in renal arterioles and other arterioles by EETs was determined to be due to a direct action on vascular smooth muscle cells.[23,32,33,34] Cell signaling mechanisms responsible for EET EDHF dilation include vascular smooth muscle cell protein kinase A (PKA) activating large-conductance K^+ (BKCa) channels to cause hyperpolarization.[38,39] Another important aspect of EET vascular action is interactions with hormonal and paracrine vasodilators and vasoconstrictors. Vasodilation by bradykinin depends on endothelial EET release.[33,40] Importantly, an EET contribution to bradykinin vasodilation has been verified in humans.[41,42] EETs also contribute to the bradykinin-dependent vascular actions under conditions of angiotensin converting enzyme (ACE) inhibition.[43,44] EETs also oppose the vasoconstrictor actions of endothelin and angiotensin II.[45,46] The findings that EETs act as EDHFs and oppose vasoconstrictors involved in renal and cardiovascular diseases provided impetus for testing if increasing EETs could provide beneficial actions in these disease states.

Renal epithelial EET actions promote natriuresis to maintain water and electrolyte homeostasis (Figure 1). EETs have been demonstrated to have epithelial actions on proximal and distal tubules.[24,47] EETs have been demonstrated to have epithelial actions on proximal and distal tubules.[48,49,50,51] Initial studies found that EETs inhibit the proximal tubule Na^+-K^+ ATPase.[49,52] EETs were also demonstrated to mediate the angiotensin II decrease Na^+/H^+ exchange in proximal tubule cells.[49] Early studies on distal nephron segments demonstrated that 5,6-EET inhibited apical sodium transport in collecting duct cells.[53] These 5,6-EET epithelial actions were COX-dependent similarly to 5,6-EET's vascular actions.[53] More recently, experimental studies have focused on the significant collecting duct epithelial actions of 11,12-EET.[50,51,54] 11,12-EET induces natriuresis through extracellular signal regulated kinase (ERK1/2)-dependent effects on the collecting duct epithelial sodium channel (ENaC).[54] Although 11,12-EET has been consistently demonstrated to inhibit ENaC using electrophysiological approaches, 14,15-EET was inactive when evaluated in isolated rat collecting ducts but 14,15-EET inhibited ENaC when evaluated in immortalized mpk-CCDc14 collecting duct cells.[48,51] Epithelial basolateral inward rectifying K^+ channels located along the convoluted tubule and collecting duct are inhibited by EETs.[47,55] 11,12-EET inhibits the basolateral K^+ channels resulting in cell membrane depolarization and reduction in the driving force for apical Na^+ reabsorption.[55]

11,12-EET could contribute to renal K^+ secretion by stimulating epithelial collecting duct principal cell BKCa channels.[56] Consequently, EETs renal epithelial actions are important in the regulation of plasma Na^+ and K^+ levels to maintain fluid homeostasis and blood pressure.

Anti-inflammatory EET actions are critical in combating hypertension and progressive kidney diseases (Figure 1). Initial studies described the ability for 11,12-EET to decrease adherent mononuclear cells in mice carotid arteries after TNFα administration[57]. Comparison between 11,12-EET to 14,15-EET and VCAM-1 blocking antibody demonstrated that 11,12-EET was superior to 14,15-EET and was as effective as VCAM-1 blocking antibody in decreasing adherent inflammatory cells.[57] 11,12-EET was also found to decrease endothelial cell VCAM-1 expression and NFκB promoter activity.[57] In rat pulmonary artery endothelial cell 11,12-EET and 14,15-EET suppressed oxidized-LDL leukotriene B4 production and activity through inhibiting p38 MAPK phosphorylation NFκB activation and inhibiting 5-lipoxygenase (5-LO).[58] 11,12-EET also opposes lipopolysaccharide induced M1 macrophage polarization and pro-inflammatory cytokines at the transcriptional and post-transcriptional level.[59] Lung inflammation during ischemia reperfusion injury or induced by cigarette smoke is decreased by 11,12-EET or 14,15-EET.[60,61] EETs act by decreasing cytokines via inhibiting NFκB activation and increasing anti-inflammatory proteins Nrf2 and heme oxygenase-1 (HO-1).[61] These anti-inflammatory actions support the notion that increasing EET levels could combat those cardiovascular and renal diseases which have a significant inflammatory contribution.

The vascular, renal epithelial, and inflammatory biological actions supported the overarching idea that decreased EETs could contribute significantly to hypertension, vascular inflammatory diseases, and acute and progressive kidney diseases (Figure 2). There have been several studies that link vascular dysfunction, inflammation, and impaired natriuresis to salt-sensitive hypertension.[15,24,47] For example, the vasoconstrictor agents can induce renal microvascular and interstitial inflammation to impair epithelial transport and sodium excretion.[15,24] Angiotensin II hypertension is salt-sensitive. Remarkably, anti-inflammatory agents improve renal microvascular function, prevent the renal interstitial inflammatory response, and eliminate salt-sensitive hypertension.[18,19] Interestingly, EET generation and regulation by sEH at the endothelial cell, epithelial cell, and inflammatory cell level could influence renal and cardiovascular function and blood pressure regulation.[23,25] Interactions between inflammation, vascular function, and epithelial sodium transport can be regulated by EETs.[23,25] In IgA nephropathy the level of renal epithelial cell sEH expression positively correlates with proteinuria and macrophage infiltration.[62] Likewise, decreased endothelial cell EET production causes dysfunction and contributes to infiltration of inflammatory cells into the vascular smooth muscle cells.[25,63] Macrophages also have the ability to generate EETs and reduced macrophage EET generation results in a profibrotic macrophage transcriptome.[64] Several studies in animal disease models and humans have consistently found that decreased EETs or increased sEH activity contributes to hypertension and the progression of cardiovascular and renal diseases.[23,24] Therefore, decreases in EET generation or increases in sEH activity can impact vascular and kidney function as well as the inflammatory state in a manner linked to water and electrolyte homeostasis and blood pressure control.

SOLUBLE EPOXIDE HYDROLASE INHIBITION

The concept that increased sEH activity converting EETs to DHETs could contribute to hypertension and salt-sensitivity accelerated the therapeutic development of sEH inhibitors (Figure 2). Human studies revealed that genetic variants in EPHX2 the gene that encodes for sEH can influence endothelial function.[65,66,67,68] The EPHX2 Arg55 genetic variant results in increased sEH activity and reduces human forearm blood flow responses to bradykinin.[68] In addition, this EPHX2 Arg55 polymorphism is associated with greater risks for coronary artery disease.[67] Acute kidney disease following cardiac surgery is associated with the EPHX2 Arg 55 polymorphism and a decreased plasma epoxide to diol ratio.[69] Endothelial dysfunction in humans has been found in instances where capacity for inducing EET release is impaired.[66] These findings in humans showed that increased sEH activity (and decreased EETs) contribute to endothelial dysfunction which is a precursor of salt-sensitive hypertension.

Evidence for epoxygenase CYP2C and CYP2J genetic variants that decrease EET and hypertension are inconclusive.[66,70,71,72] Several CYP2C and CYP2J2 gene variants demonstrate reduced epoxygenase activity.[66,70,71,72] As an example, CYPJ2 gene G-50 single nucleotide polymorphism is associated with increased coronary artery disease risk and decreased plasma EET levels.[70] Analyses of ethnic cohorts have demonstrated no association with hypertension or an increased risk for essential hypertension.[71,72,73,74] On the other hand, animal studies on salt regulation and blood pressure control have consistently demonstrated that an impaired ability to increase CYP2C generation of EETs in response to dietary NaCl contributes to salt-sensitive hypertension.[24,47,54] Decreased renal epoxygenase activity and EETs have been associated with hypertension in angiotensin hypertension and Lyon hypertensive rats.[75,76,77] Cyp2c44 −/− and Cyp4a10 −/− mice exhibit decreased renal epoxygenase activity in response to a high salt diet and develop salt-sensitive hypertension (Figure 2).[48,54,78] These findings provided compelling reasons for developing sEH inhibitors as a means to increase renal EET levels and to treat salt-sensitive hypertension and cardiovascular diseases.

Initial studies determining kidney CYP2C epoxygenase and sEH regulation were conducted in angiotensin II hypertension.[75,76,79,80] These studies discovered that angiotensin II hypertension was associated with an increased kidney sEH expression that resulted in a decrease in the EET to DHET ratio.[75,79] In contrast, rat angiotensin II salt-sensitive hypertension did not have a change in sEH expression but did have an inability to increase kidney and renal microvascular CYP2C expression in response to a high salt diet.[76] The decrease in CYP2C expression was also found to be partly responsible for the persistence of salt-sensitive hypertension following cessation of angiotensin II infusion.[76] Thus, these experimental studies support the notion that the increase in kidney CYP2C expression in response to a high salt diet in normotensive rats contributes to water and electrolyte homeostasis and blood pressure control. In addition, inhibiting sEH to increase EETs in angiotensin II and salt-sensitive hypertension improved natriuresis, reduced afferent arteriolar responses to angiotensin II, improved endothelial-dependent dilation, decreased renal inflammation, and slowed progressive kidney damage.[76,79,80] Thus, the evaluation of

sEH inhibitors supported the notion that increasing kidney EET levels had therapeutic potential in hypertension.[81]

The development of sEH inhibitors progressed rapidly due to extensive knowledge concerning the enzymatic binding and activity, the ability to synthesize carbamate urea sEH inhibitors in large quantities, and development of the first orally active sEH inhibitor, AUDA (Table 1).[81] Current orally active sEH inhibitor structures include urea-based sEH inhibitors, piperidyl urea sEH inhibitors, an amide series of sEH inhibitors and animoheteroarlyl sEH inhibitors.[81,82,83] Experimental studies with sEH inhibitors determined anti-hypertensive actions in in a number of models: spontaneously hypertensive rats (SHR), angiotensin II hypertension, DOCA-salt hypertension, Lyon hypertensive rats, and Ren-2 transgenic hypertension.[75,84,85,86] These studies included sEH inhibitors administered in preventive and clinically relevant interventional manner to rat and mice hypertension models. A consistent finding was that sEH inhibitors lowered blood pressure in these hypertension animals (Figure 2). EPHX2 gene deletion resulted in decreased blood pressure in DOCA-salt hypertension and sEH inhibitors did not have additional actions in EPHX2 gene deleted mice.[85] The decrease in blood pressure in response to sEH inhibitors or genetic EPHX2 deletion was associated with an increase in the EET to DHET ratio, natriuresis, improved renal microvascular function, and decreased renal inflammation.[85] Interestingly, in several instances the decrease in kidney damage following chronic sEH inhibition was greater than what would be expected based on the degree of blood pressure lowering.[79,85,87] Subsequent studies found that sEH inhibitors could decrease progressive kidney disease independent of blood pressure lowering actions.[88,89,90] Findings in these hypertension animal models resulted in extensive investigation of sEH inhibitors to treat renal and cardiovascular diseases.

Kidney-protective actions of sEH inhibitors have been demonstrated to be independent of the anti-hypertensive actions. Diabetic and hypertensive kidney injury has been clearly demonstrated to be decreased by the sEH inhibitor AUDA in the Goto-Kakizaki diabetic rat.[88] The decrease in renal inflammation, glomerular injury, and tubular damage in the Goto-Kakizaki rats treated with an sEH inhibitor was not associated with a lowering of blood pressure, blood glucose, cholesterol, or triglyceride levels.[88] Kidney-protective actions of sEH inhibitors or EPHX2 genetic deletion have also been demonstrated in unilateral ureter obstruction (UUO) induced kidney fibrosis.[89,90] Administration of sEH inhibitors or EPHX2 genetic deletion decreased kidney inflammation by decreasing neutrophil infiltration and decreasing cytokine TNFα and ICAM-1 levels.[89,90] The anti-inflammatory sEH inhibitor cell signalling mechanisms of actions in UUO were determined to be via NFκB downregulation and decreased transforming growth factor-β1 (TGF-β1)/Smad 3 mediated inflammation.[89,90] Additional evaluation revealed that sEH inhibition anti-fibrotic actions were due to suppressing the epithelial to mesenchymal transition.[89,90] Renal epithelial cell culture studies determined that sEH inhibition increases E-cadherin resulting in decreased α-smooth muscle actin to prevent transition from an epithelial cell to myofibroblast cell phenotype.[89,90] Similar results were obtained in drug-induced nephrotoxicity studies.[91,92] Administration of sEH inhibitors decreased NFκB activation and TNFα inflammation to reduce cisplatin induced acute kidney injury.[91] Taken together, these studies demonstrated

that sEH inhibitors decrease progressive renal disease through anti-inflammatory and anti-fibrotic mechanisms (Figure 2).

Cardiovascular diseases represent another area for which sEH inhibitors hold an exciting promise. Left ventricular cardiac hypertrophy and cardiac ischemia reperfusion injury are two animal disease models where sEH inhibitors have been extensively tested.[25] Decreased EET generation and increased sEH activity is evident in several rodent cardiac hypertrophy models.[93,94,95] Administration of sEH inhibitors prevents and reverses pressure overload left ventricular hypertrophy.[93,96,97] Angiotensin II hypertension mediated cardiac hypertrophy is attenuated by sEH inhibition and the decrease in hypertrophy is partially independent of blood pressure lowering.[93,98] Decreased cardiac hypertrophy in response to sEH inhibition is mediated by anti-inflammatory and anti-fibrotic actions.[95,99] Like left ventricular hypertrophy, myocardial infarction is also improved by sEH inhibitors.[100,101,102] Several dog, rat, and mice studies have demonstrated positive short-term and long-term outcomes for sEH inhibitors to treat cardiac ischemia reperfusion injury.[99] The short-term positive effects on cardiac ischemia reperfusion injury are mediated through K_{ATP} channel activation, PI3 kinase signalling, and delays mitochondrial permeability transition pore (mPTP) opening to oppose cardiac cell apoptosis and necrosis.[103,104,105] The long-term cardiac fibrotic and hypertrophy following myocardial infarction is also reduced by sEH inhibitors.[99,105] Atrial fibrillation and cardiac arrhythmias in ischemia reperfusion and cardiac hypertrophy animal models are attenuated sEH inhibitors.[99,106,107] These long-term cardiac protective actions for sEH inhibitors have significant anti-inflammatory actions.[99] Treatment with sEH inhibitors decreases circulating inflammatory cytokines and inhibits cardiac NFκB activation.[96,108] Likewise, chronic sEH inhibitor administration decreases cardiac fibrosis and hypertrophy by decreasing MAPK and endoplasmic reticulum stress activation.[99,109] The sEH inhibition cerebral protective effects from stroke also involve reduction in MAPK and PI3 kinase signaling to prevent neuronal cell apoptosis.[110,111,112] Intriguingly, sEH inhibitor anti-inflammatory actions are a major mechanism for protective actions in vascular diseases.[25] The increase in cytokines, chemokines, and adhesion molecules that occurs in atherosclerotic and vascular remodeling animal models is greatly reduced by sEH inhibition.[113,114,115] More recently, sEH inhibition demonstrated anti-inflammatory coronary artery actions in a Kawasaki disease mouse model that is an acquired heart disease in pediatric patients.[116] These finding provide evidence for broad cardiovascular protective actions for sEH inhibitors that utilize EET anti-inflammatory, anti-apoptotic, and anti-fibrotic actions (Figure 2).

The overwhelming body of evidence from preclinical animal models for sEH inhibition having therapeutic value in hypertension, renal diseases, and cardiovascular diseases has allowed for rapid advancement of sEH inhibitors to clinical trials. Two sEH inhibitors, AR9281 and GSK2256294, have advanced to human clinical trials.[25,27,79] Another sEH inhibitor, EC5026, is advancing to a Phase 1 clinical trial for chronic pain management. Phase I clinical trials for AR9281 and GSK2256294 were positive for safety and pharmacokinetics.[117,118] AR9281 entered into a Phase IIa clinical trial for hypertension and type 2 diabetes but did not provide sufficient efficacy.[81] Although GSK2256294 has not advanced to Phase II clinical trials, there were positive cardiovascular findings. Endothelial function assessed by forearm blood flow responses to bradykinin was improved in male

obese smokers.[27] This human study confirms the sEH inhibitor cardiovascular beneficial actions observed in copious animal studies. There are two human clinical trials that are in the recruiting and beginning stages for evaluating the sEH inhibitor GSK2256294 for treatment of insulin resistance and subarachnoid hemorrhage. There appears to be a bright and exciting future for sEH inhibitors to one day be approved for treating human disease.

EET ANALOGS

Given the successful development of the sEH inhibitors to clinical trials, one might ask what the benefit would be for developing EET analogs to target the epoxygenase pathway. There are three primary reasons to develop EET analogs. The first reason is that sEH inhibitors could be less effective in situations where decreased EET levels are due to decreased CYP2C epoxygenase EET generation. As previously mentioned, decreased CYP2C generation has been found in animals with salt-sensitive hypertension.[76] Administration of sEH inhibitors to these salt-sensitive hypertension animals are not always as effective in lowering blood pressure.[75,88] A second reason for developing EET analogs is that sEH inhibition will increase epoxy fatty acids in general rather than specific EETs.[23,81] The contribution of epoxy fatty acids such as linoleic epoxy fatty acids to the beneficial effects for sEH inhibitors has not been extensively evaluated. EET analogs represent a direct method to mimic arachidonic acid epoxy fatty acids (EETs).[25,26] The third reason is the possibility of developing EET analogs that resist metabolism by sEH and other pathways.[25,26] Several biosteres that mimic epoxide bonds, replace the carboxylic acid and mimic double bonds present in 11,12-EET and 14,15-EET result in EET analogs that retain biological activity and resist metabolism (Table 1).[25,26] The last reason for developing EET analogs is that regiospecific 11,12-EET and 14,15-EET mimics can be developed. There are four regioisomeric EETs and in several circumstances these EETs have variable biological actions.[23,29,47] In addition, the sEH preferred regioisomeric EET substrate is 14,15-EET with lesser preference for 11,12-EET and 8,9-EET and 5,6-EET as a poor substrate for sEH.[23,81] Kidney glomerular permeability studies provides evidence where the EET regioisomeric analog action is important. Focal segmental glomerular sclerosis circulating factor or angiotensin II induced increases in glomerular permeability are blocked by 8,9-EET and 8,9-EET analogs.[119,120] In contrast, by 5,6-EET, 11,12-EET, 14,15-EET, 11,12-EET analogs and 14,15-EET analogs are ineffective in blocking the increase in glomerular permeability.[119] Consequently, EET regioisomeric analogs have been developed and evaluated in preclinical animal models for renal and cardiovascular diseases.

Like sEH inhibitors, EET analogs were developed and initially tested in hypertension and salt-sensitive hypertension.[121,122] Genetic manipulation to increase EET levels was evaluated prior to testing EET analogs in salt-sensitive hypertension. Mice with endothelial-specific overexpression of the human CYP2J2 (*Tie2*-CYP2J2) and CYP2C8 (*Tie2*-CYP2C8) epoxygenases showed attenuated afferent arteriole constrictor responses to endothelin-1 and enhanced dilator responses to acetylcholine.[45] Angiotensin II salt-sensitive hypertension and renal injury was significantly attenuated in Tie2-*CYP2J2* and Tie2-*CYP2C8* mice.[45] Importantly, we found that primary renal endothelial cells isolated from Tie2-*CYP2J2* and Tie2-*CYP2C8* mice produced significantly higher concentrations of 11,12-EET and 14,15-EET compared to renal endothelial cells isolated from wild-type controls.[45] The subsequent

development of orally bioavailable EET analogs allowed for testing in rodent hypertension. EET analogs for 11,12-EET and 14,15-EET lowered blood pressure in SHR, angiotensin hypertension, and salt-sensitive hypertension.[121,122] The 11,12-EET analog, NUDSA, was also demonstrated to decrease blood pressure and improve endothelial function in mice with metabolic syndrome.[123] Likewise, 14,15-EET analogs, EET-A and EET-B, decreased blood pressure in angiotensin hypertension.[121,122] Additional studies in angiotensin hypertension revealed that EET-A improved mesenteric resistance artery endothelial function and inhibited ENaC to promote sodium excretion.[122] Malignant angiotensin hypertension in Cyp1a1-Ren transgenic rats was also prevented by chronic EET-A administration.[124] EET analogs also decreased renal inflammation and macrophage infiltration in hypertension.[122] EET analogs are anti-hypertensive and improve renal function through HO-1 signalling and inhibition of the sodium-chloride transporter (NCC).[125] Hypertension resulting from cyclosporine administration to rats for one month was prevented by EET analog treatment.[126] EET-B treatment decreased renal inflammation, apoptosis, and fibrosis associated with cyclosporine.[126] Cyp2c44 −/− mice have been extensively studied because Cyp2c44 is the major kidney epoxygenase and Cyp2c44 expression is increased in response to a high K^+ or high Na^+ salt diet to generate EETs in the cortical collecting duct.[47] More importantly, mice with Cyp2c44 epoxygenase genetic deficiency and decreased EET levels develop salt sensitive hypertension.[54,122] Hyperactive ENaC and a reduction in ERK1/2 ENaC subunit phosphorylation contribute to the salt-sensitive hypertension in Cyp2c44 −/− mice.[54] EET-A prevented salt-sensitive hypertension and decreased renal injury in Cyp2c44 −/− mice.[122] The anti-hypertensive actions of EET-A in Cyp2c44 −/− mice were likely a result of the ability for EET-A to inhibit ENaC in cortical collecting duct cells.[122] These findings support the notion that EET analogs lower blood pressure in hypertension through the known EET vascular, epithelial transport, and anti-inflammatory actions (Figure 2).

Renal protective actions have been demonstrated for EET analogs that are independent of systemic actions.[25] Acute and chronic renal disease animal models provide evidence that EET analogs anti-inflammatory, anti-fibrotic, and anti-apoptotic actions decrease renal disease progression.[25] EET analogs administered in a preventive or interventional mode can decrease renal vascular, glomerular, and tubular injury.[25] Initial studies evaluated EET analogs in drug-induced nephrotoxicity.[127] Treatment with EET analogs decreased the tubular injury in response to the chemotherapeutic agent cisplatin.[127] Cisplatin induced nephrotoxicity renal inflammation and apoptosis was reduced by EET analog treatment.[127] Renal ischemia reperfusion injury is decreased by EET analog treatment.[128] EET analog treatment improved renal oxygenation, decreased inflammatory cell infiltration, and acted via PI3 kinase Akt, GSK-3β signalling to reduce epithelial cell apoptosis.[128] EET analogs also protect from long-term progressive renal injury.[25] Kidney fibrosis in UUO mice is prevented by EET-A through actions on epithelial to mesenchymal transition.[129] EET-A treatment to UUO mice resulted in a decrease in epithelial to mesenchymal transducers and myofibroblast markers resulting in decreased renal α-smooth muscle actin and collagen levels.[129] Progressive renal injury due to radiation exposure is also prevented by EET analogs.[130] EET-A given two days following radiation exposure to rats mitigated the progressive renal microvascular and tubular damage.[130] The decrease in radiation induced renal damage by EET-A was associated with reductions in the p53/Fas/FasL apoptotic

pathway.[130] Likewise, renal injury associated with lupus nephritis is decreased by EET analog treatment.[131] Systemic lupus nephritis mice treated with EET-A had markedly diminished CXC chemokines and receptors, reduced renal TNF-α, IL-6, and IL-1β levels, and reduced renal immune cell infiltration resulting in decreased renal damage.[131] As a whole, EET analogs have been consistently demonstrated to decrease acute and chronic kidney disease progression through anti-apoptotic, anti-inflammatory, and anti-fibrotic mechanisms (Figure 2).

EET analogs cardiovascular protective actions have been evaluated in several cardiac disease animal models.[25] Initial studies were conducted with EET analogs in cardiac ischemia reperfusion injury.[132,133,134] These studies revealed that NUDSA administered after myocardial infarction resulted in improved cardiac contractile function.[134] Likewise, left ventricular function one month following left anterior descending coronary artery ligation in mice was improved by EET analog treatment started five days after ligation.[134] EET-B treatment for two months improved cardiac function in SHR subjected to 30 minutes of left coronary artery occlusion to induce myocardial infarction.[133] The improved cardiac function in SHR was linked to increased HO-1 cardiomyocyte levels and decreased inflammation and fibrosis.[133] Obesity-induced diabetic cardiomyopathy in mice was reduced by EET analog treatment.[135] EET analog administration resulted in peroxisome proliferator-activated receptor coactivator (PGC-1α) induction to control mitochondrial function, induce HO-1, and reduce inflammation in the heart of obese diabetic mice.[131] A contribution of EET activation in heart vascular endothelial cells to cardiac protection has been demonstrated in endothelial specific CYP2J2 overexpressing mice.[136] Myocardial perfusion following myocardial infarction in mice was increased by increased endothelial cell EET levels.[136] Endothelial cell EETs increased angiogenesis via Jagged1/Notch 1 signalling in mice following cardiac ischemia.[136] Cardiac hypertrophy was also decreased in hypertensive rats treated with EET-A for two weeks.[137,138] Ischemia reperfusion induced ventricular fibrillation in hypertension was also greatly diminished by EET analogs.[138] The primary signalling mechanisms that appear to mediate EET analog cardiac protective actions include opposing apoptosis and improving mitochondrial function.[25,132,139] In line with EET biological actions, EET analogs activate cardiac myocyte mitochondrial K_{ATP} channels resulting in an increase in mitochondrial cristae density.[99,140] Anti-apoptotic EET analog actions are mediated through MAP kinase signalling in the heart to prevent ischemia reperfusion injury.[25,99] These findings support the notion that EET analogs are a potential therapeutic strategy for cardiac diseases (Figure 2).

FUTURE FOR EPOXY FATTY ACID THERAPEUTICS

We have made significant strides on the journey to developing EET-based therapeutics for treating renal and cardiovascular diseases. We have gone from the initial description of EETs as regulators of renal hemodynamics and epithelial transport to the development of sEH inhibitors and EET analogs. In less than a decade sEH inhibitors were in clinal trials for hypertension and diabetes.[81] On the other hand, EET analogs developed at a slower pace due to the lack of a protein target and ability to obtain orally bioavailable EET analogs.[26] EET analogs are now being advanced towards clinical trials for kidney diseases. The journey

continues as the potential for an EET pathway drug being approved for treating renal or cardiovascular diseases appears likely.

Challenges and headwinds still exist for epoxy fatty acids to reach a point to benefit human health. As with every hormonal and paracrine factor and cell signalling pathway, there is an upside and downside to therapeutically targeting EETs and sEH. Although the positives appear to outweigh the negatives for sEH inhibitors and EET analogs, there has been concerns with their potential due to effects on angiogenesis and tumor growth and metastasis in certain cancers.[141,142,143] In contrast, however, sEH inhibitors given alone or in combination with COX inhibitors can have anti-cancer actions.[144,145,146] EET angiogenesis could be a detrimental action for tumors but be very useful when increased vascularization of ischemic tissue is required.[147,148] In addition, the EET actions on the pulmonary vasculature is vasoconstriction.[149,150] This opposite pulmonary vascular EET actions is like that found for prostaglandins.[149] Even with these potential challenges, sEH inhibitors have demonstrated beneficial cardiovascular effects in human clinical trials to combat chronic diseases.[27] Opportunities for therapeutically targeting epoxy fatty acids remain abundant.

Dietary factors like diets enriched with ω−3 fatty acids can influence ω−3 epoxy fatty acid levels and consumption of specific plants can influence sEH activity. There has been considerable investigation into the actions for ω−3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) fatty acids. Increases in dietary ω−3 EPA and DHA have been found to have cardiovascular beneficial actions.[151,152] Intriguingly, epoxygenase enzymes generated EPA epoxyeicosatetraenoic acids (EEQs) and DHA epoxydocosapentaenoic acids (EDPs) metabolites that have also been found to have kidney and cardiovascular actions.[153,154,155,156] Interestingly, 17,18-EEQ analogs have been developed, tested, and reached Phase 1 clinical trials for treating atrial fibrillation and cardiac arrhythmias.[152,154] Other dietary means can elevate epoxy fatty acids. Plants from the order Brassicales and Cimicifuga dahurica roots have been found to have decrease sEH activity.[157,158] Eating flaxseed has been demonstrated to inhibit sEH and lower blood pressure in humans with hypertension.[159] There is great potential and much to be explored for the kidney and cardiovascular dietary ω−3 epoxy fatty acids and plants with sEH inhibitory activity.

Another major area open for opportunities are to better define the contribution for EET regioisomers. 11,12-EET and 14,15-EET have been the most extensively studied EET regioisomers; however, 5,6-EET and 8,9-EET have been demonstrated to have vascular and kidney actions.[23,25] Vascular endothelial cell actions have been described for 5,6-EET to activate transient receptor potential vanilloid receptor 4 (TRPV4) channels.[160] An EET-binding pocket mediates 5,6-EET TRPV4 activation and can control hypotonic cell swelling.[160] Likewise, 8,9-EET and 8,9-EET analogs have selective actions on glomerular permeability induced by a circulating permeability factor.[119] Regioisomeric actions on flow induced arteriolar dilation have been determined for with 11,12-EET and 14,15-EET selective antagonists.[161] These studies revealed that 14,15-EET but not 11,12-EET contributed to mesenteric resistance artery flow induced dilation.[161] Interestingly, decreased 14,15-EET levels are significantly associated with abdominal aortic calcification in patients with primary aldosteronism.[162] Another interesting aspect for EETs is that there is significant evidence for the existence of EET receptors.[163,164] Given the difference in EET

regioisomeric renal and cardiovascular actions and the potential for EET receptors, there are still significant gaps in knowledge pertaining to EETs that require experimental investigation.

Bifunctional molecules that alter epoxy fatty acids is another emerging area for further development and exploration.[165,166] The development of bifunctional molecules has revolved around having sEH inhibition as a focal activity for the molecules (Table 1).[165,167] EET analogs capable of inhibiting sEH are an example of fused pharmacophore dual modulators.[25,168] Although these have been described based on *in vitro* effects on vascular function, there has been no significant evaluation of these bifunctional molecules in animal disease models. One of the first dual modulators was a linked pharmacophore with combined sEH and COX-2 inhibition.[145,167] The dual sEH and COX-2 inhibitor, PTUPB combats diabetic kidney injury in Zucker Diabetic Fatty rats.[167] In addition, PTUPB has been demonstrated to have anti-cancer and anti-metastatic actions in mice.[145,146] Finally, merged pharmacophore sEH inhibitors with nuclear receptor agonism have demonstrated great potential. RB394 is a dual sEH inhibitor and PPARγ agonist that has been demonstrated to combat diabetes and associated liver and kidney injury.[169] Another merged pharmacophore combines sEH inhibition with FXR agonism.[170] The dual acting sEH inhibitor and FXR agonist, DM509 has been demonstrated to combat non-alcoholic liver disease to a greater extent than an FXR agonist given alone.[170] These findings demonstrate that bifunctional molecules with sEH inhibitor activity could be used to treat heart, kidney, liver, and metabolic diseases.

CONCLUSION

Epoxy fatty acids contribution to renal, vascular, and heart function led to the notion that increasing EETs could combat disease. EETs were initially found to be vasodilatory and natriuretic; their importance in salt-sensitive hypertension was clearly established. The combination of pharmacological and genetic tools determined the importance for the EETs and sEH in hypertension, diabetes, ischemic kidney and heart diseases, stroke, and others. Human studies provided additional support to the importance of the epoxygenase pathway in these diseases. Development of sEH inhibitors and EET analogs has progressed to a point where their exciting therapeutic potential to treat kidney and cardiovascular diseases is being actively investigated.

ACKNOWLEDGEMENTS

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SOURCE OF FUNDING

This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases DK103616 and Dr. Ralph and Marian Falk Medical Research Trust Bank of America, N.A., Trustee.

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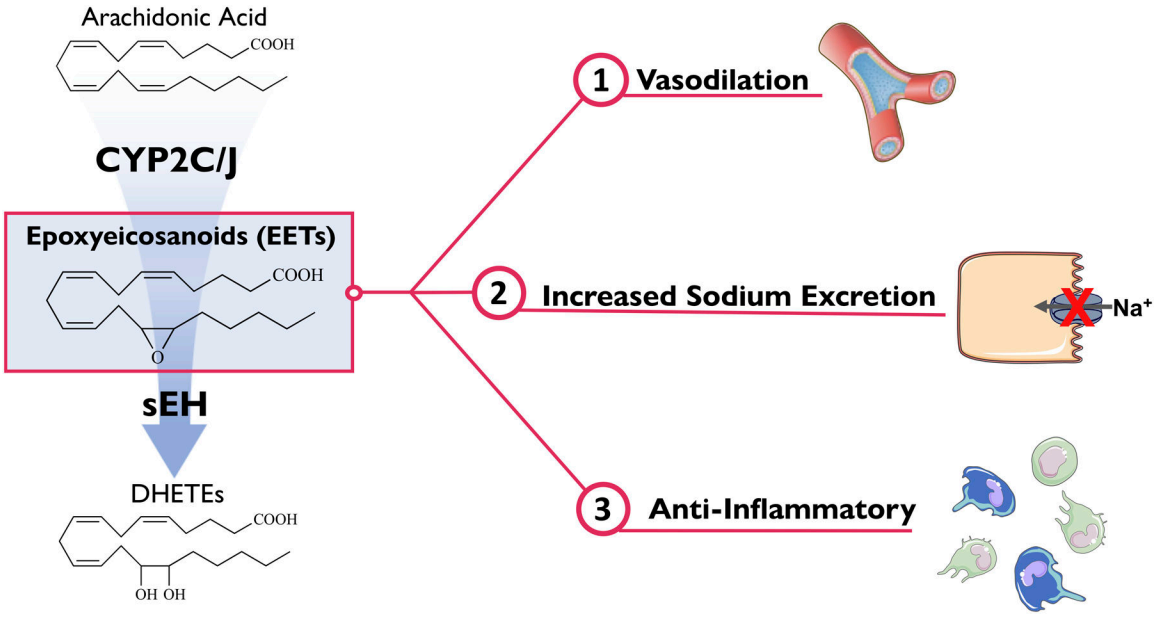


Figure 1.

Epoxyeicosanoids (EETs) cause vasodilation, increased sodium excretion, and are anti-inflammatory.

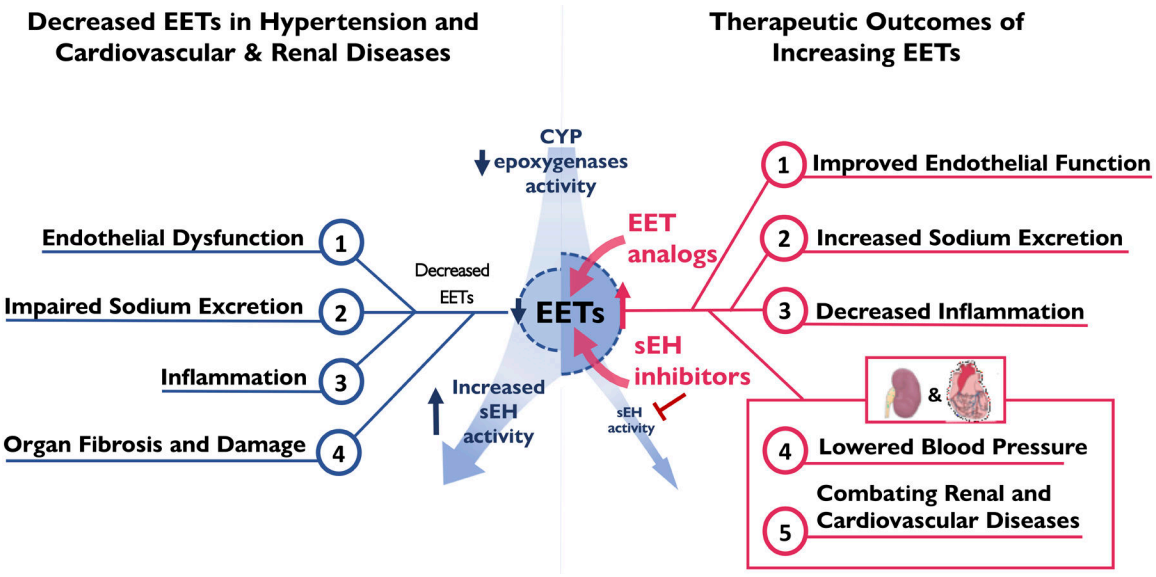


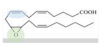
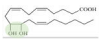
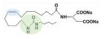
Figure 2. Left Panel:

Decreased CYP epoxygenase activity or increased sEH activity results in decreased epoxyeicosatrienoic acids (EETs) resulting in vascular dysfunction, inflammation, impaired pressure natriuresis, organ fibrosis, and hypertension mediated renal and cardiovascular (CV) complications.

Right Panel: Epoxyeicosatrienoic acid (EET) analogs and soluble epoxide hydrolase (sEH) improve vascular function, decrease inflammation, increase natriuresis, combat cardiovascular (CV) diseases, lower blood pressure, combat kidney diseases.

Table 1.

Epoxy fatty acid and therapeutic structures

Classes of Eicosanoid Molecules	Molecules	Structure Activity Relationship		References
Regioisomeric Epoxyeicosatrienoic Acids (EETs) Dihydroxyeicosatrienoic Acids (DHETs)	5,6-EET 8,9-EET 11,12-EET 14,15-EET 5,6-DHET 8,9-DHET 11,12-DHET 14,15-DHET	EETs have an epoxide bond (11,12-EET, green) and require at least one carbon double bond (blue).		34–39, 46, 49–61, 117, 158
		DHETs are sEH products of epoxide bond converted to diol (11,12-DHET, green).		
Soluble Epoxide Hydrolase (sEH) Inhibitors	AUDA AR9281 GSK2256294 EC5026	AUDA is a urea-based sEH inhibitor (green). sEH inhibitors include amide, piperidyl, and aminoheteroaryl inhibitors.		82–91, 94–96, 98–101, 103–109, 111–116, 142, 146
EET Analogs	NUDSA EET-A EET-B	EET-A is 14,15-EET analog with a urea (green) that mimics the epoxide and resist she activity and a double bond at the 8,9 position (blue).		119–133, 135, 166
Dual Modulator Soluble Epoxide Hydrolase (sEH) Inhibitors	PTUPB RB394 DM509	PTUPB is a dual sEH inhibitor (green) and COX-2 inhibitor (blue) with a linker.		143, 144, 165, 167, 168