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INVITED REVIEW

Epoxyeicosanoids in Hypertension

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Summary

Epoxyeicosatrienoic acids (EETs) are also known as epoxyeicosanoids that have renal and cardiovascular actions. These renal and cardiovascular actions can be regulated by soluble epoxide hydrolase (sEH) that degrades and inactivates EETs. Extensive animal hypertension studies have determined that vascular, epithelial transport, and anti-inflammatory actions of EETs lower blood pressure and decrease renal and cardiovascular disease progression. Human studies have also supported the notion that increasing EET levels in hypertension could be beneficial. Pharmacological and genetic approaches to increase epoxyeicosanoids in several animal models and humans have found improved endothelial vascular function, increased sodium excretion, and decreased inflammation to oppose hypertension and associated renal and cardiovascular complications. These compelling outcomes support the concept that increasing epoxyeicosanoids *via* sEH inhibitors or EET analogs could be a valuable hypertension treatment.

Key words

Epoxyeicosatrienoic acid • Natriuresis • Endothelium • Sodium channels

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Introduction

Hypertension is the most prevalent cardiovascular disease that afflicts one in every three adults worldwide (Barri 2008, Mills *et al*. 2016). Several factors contribute to the chronic blood pressure elevation which increases the risk for cardiovascular morbidity and mortality. Contributing factors to hypertension include an elevated renin-angiotensin system, increased sympathetic activity, and inflammation (Imig *et al*. 2018, Kaplan 2016). These factors result in excessive vasoconstriction and increased total peripheral resistance or impaired sodium excretion, increased extracellular fluid volume, and increased cardiac output (Imig *et al*. 2018). Antihypertensive drugs counteract these contributing factors and include renin-angiotensin system inhibitors, β-blockers, vasodilators, and diuretics (Chobanian 2003, Kaplan 2016). These therapies have been largely successful in lowering blood pressure in hypertension; however, evidence suggests that antihypertensive treatment is suboptimal since cardiovascular morbidity and mortality remains a significant health care problem (Chobanian *et al*. 2003, Kaplan 2016, Munter *et al*. 2014). Research continues to identify novel contributing factors to blood pressure regulation that could improve hypertension treatment and decrease cardiovascular mortality. Contributing factors to blood pressure regulation that have garnered significant attention are the epoxy fatty acids, epoxyeicosatrienoic acids (EETs) and their metabolism by soluble epoxide hydrolase (sEH) (Capdevila and Wang 2013, Imig 2012).

EETs are twenty carbon epoxy fatty acids also known as epoxyeicosanoids. EETs are generated from arachidonic acid by cytochrome (CYP) P450 epoxygenases. CYP2C and CYP2J enzymes produce four regioisomeric EETs; 5,6-EET, 8,9-EET, 11,12-EET, and

14,15-EET (Capdevila and Wang 2013, Imig 2012). EETs are produced and have activities in endothelial cells, kidney, heart, and other organs that impact blood pressure regulation (Capdevila and Wang 2013, Imig 2012). Blood pressure regulating EET actions include vasodilation, increasing sodium excretion, and decreasing inflammation (Bellien and Joannides 2013, Imig 2015). These EETs are then metabolized by sEH to dihydroxyeicosatrienoic acids (DHETs) that have reduced or no biological activity (Fig. 1) (Imig 2012, Imig 2018). Genetic variations in the sEH gene EPHX2 that increase sEH activity have been demonstrated to cause impaired endothelial vasodilator responses in humans (Bellien and Joannides 2013, Lee *et al*. 2011). Likewise, human studies provide evidence that decreased EET levels result in an elevated blood pressure. CYP2C epoxygenase enzyme gene variants demonstrate reduced EET generation and increased risk for essential hypertension (Dreisbach *et al*. 2005, King *et al*. 2005, Polonikov *et al*. 2008, Yu *et al*. 2004,). Attractively, increasing EET levels in hypertension animal models results in blood pressure lowering and cardiovascular protective actions (Campbell *et al*. 2017, Imig and Hammock 2009).

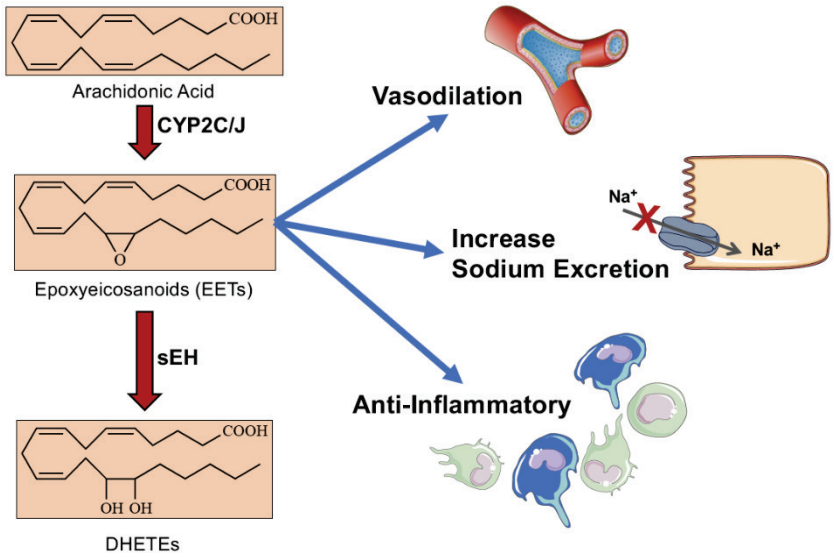

Fig. 1. Epoxyeicosanoids have vasodilator, natriuretic, and anti-inflammatory activities. Left panel illustrates the arachidonic acid metabolism by CYP2C or CYP2J epoxygenase enzymes to epoxyeicosatrienoic acids (EETs) and metabolism of EETs by soluble epoxide hydrolase (sEH) to dihydroxyeicosatrienoic acids (DHETs). Right panel illustrates epoxyeicosanoid actions to cause vasodilation, increase sodium excretion, and combat inflammation.

Epoxyeicosanoids and vascular regulation in hypertension

Excessive vasoconstriction in hypertension increases total peripheral resistance and decreases renal blood flow that results in impaired sodium excretion. Although elevated angiotensin II levels are a main contributing factor, other factors that cause excessive vasoconstriction in hypertension include elevated endothelin, increased thromboxane, decreased endothelial nitric oxide, and reduced endothelial EET levels (Imig *et al*. 2018). Reduced nitric oxide and EET levels are major reasons for endothelial dysfunction in hypertension (Bellien *et al*. 2012, Imig *et al*. 2018, Lee *et al*. 2011). Endothelial dysfunction in hypertension and other cardiovascular diseases is associated with poor health outcomes (Baylis 2012, Imig *et al*. 2018, Montezano and Touyz 2012). Extensive evidence in hypertension animal models provides support to the notion that increasing epoxyeicosanoid EETs improves endothelial function and lowers blood pressure in hypertension (Campbell *et al*. 2017, Imig 2018, Imig and Hammock 2009).

EETs are endothelial-derived hyperpolarizing factors that ensure proper resistance artery and arteriolar function (Campbell *et al*. 1996, Fissthaler *et al*. 1999, Imig *et al*. 1999). 11,12-EET and 14,15-EET are generated by the endothelium and dilate arterioles through activating vascular smooth muscle cell large-conductance calcium-activated K^+ (K_{Ca}) channels (Imig *et al*. 1999, Imig *et al*. 2008). Endothelial EET levels are decreased in several hypertension animal models (Imig *et al*. 2002, Zhao *et al*. 2003). The decrease in vascular endothelial levels is a consequence of decreased CYP2C11 and CYP2C23 expression in obese Zucker and high fat fed rats that have hypertension (Zhao *et al*. 2005). On the other hand, angiotensin-dependent hypertension is associated with increased vascular sEH expression that leads to decreased EET levels (Imig *et al*. 2002). Endothelial dysfunction in angiotensin-dependent hypertension is due to decreased vascular EET levels

(Imig *et al*. 2002). Increasing EET levels with sEH inhibition improves endothelial dysfunction in rodent obesity, metabolic syndrome and hypertension models (Campbell *et al*. 2017, Imig and Hammock 2009). Remarkably, humans that are obese smokers with chronic obstructive pulmonary disease (COPD) and treated with an sEH inhibitor for two weeks demonstrated improved endothelial function (Yang *et al.* 2017). These findings support the notion that increasing EET levels will improve endothelial function and cardiovascular morbidity and mortality in hypertension.

Hypertension, kidney function and epoxyeicosanoids

A decrease in renal epoxygenase activity has been strongly linked to hypertension including angiotensin-dependent and salt-sensitive hypertension (Imig 2018, Imig *et al*. 2002, Zhao *et al.* 2003). Rats overexpressing human renin and angiotensinogen genes (dTGR) have decreased renal epoxygenase activity and kidney CYP2C11 and CYP2C23 protein levels (Kaergel *et al.* 2002). Angiotensin-dependent hypertension is also associated with increased renal sEH protein expression (Imig *et al*. 2002). Regarding salt regulation, rodent kidney CYP2C epoxygenase enzymes are upregulated in response to a high-salt diet (Lee *et al*. 2010, Makita *et al*. 1994, Zhao *et al.* 2003). Conversely, an inability to upregulate CYP2C epoxygenases in response to a high-salt diet leads to impaired sodium excretion and salt-sensitive hypertension (Lee *et al*. 2010, Makita *et al*. 1994, Zhao *et al.* 2003). Taken together, decreased kidney EET levels impairs vascular and epithelial function and contributes to the elevated blood pressure and progression of hypertension.

Extensive evidence in hypertension and renal disease studies demonstrates an important contribution for EETs in maintaining kidney vascular and epithelial function (Capdevila and Wang 2013, Imig 2012, Imig 2018). EETs act to dilate preglomerular afferent arterioles and inhibit epithelial sodium channels (ENaC) (Campbell *et al*. 2017, Imig 2018). Decreased EET levels in hypertension leads to excessive afferent arteriolar constriction and enhanced ENaC activity and salt absorption (Fig. 2) (Capdevila *et al*. 2014, Zhao *et al*. 2003). Angiotensin-dependent hypertension has increased afferent arteriolar constrictor reactivity that contributes to impaired natriuresis (Zhao *et al*. 2003). Excessive afferent arteriolar constrictor reactivity in hypertension is eliminated by sEH inhibition to increase kidney EET levels (Zhao *et al*. 2003, Zhao *et al*. 2004). Increased ENaC in angiotensin-dependent hypertension also contributes the sodium retention and increase in blood pressure (Khan *et al*. 2014). Salt-sensitive hypertension occurs when the kidney and vascular CYP2C23 and CYP2C11 fails to increase in response to a high-salt diet (Zhao *et al*. 2003, Zhao *et al*. 2004). In accordance with these findings, genetic deletion of Cyp2C23 (Cyp2c44) in mice results in decreased kidney and vascular EET levels and salt-sensitive hypertension (Capdevila *et al*. 2014, Imig 2012, Sun *et al*. 2010). Indeed, 11,12-EET can inhibit cortical collecting duct ENaC and increase sodium excretion (Capdevila *et al*. 2014). Increasing EETs can lower blood pressure by inhibiting sodium absorption in the proximal tubule and cortical collecting duct. (Capdevila *et al*. 2014, Khan *et al*. 2014) Taken as a whole, EETs have antihypertensive actions on the renal afferent arteriole and epithelial sodium transport.

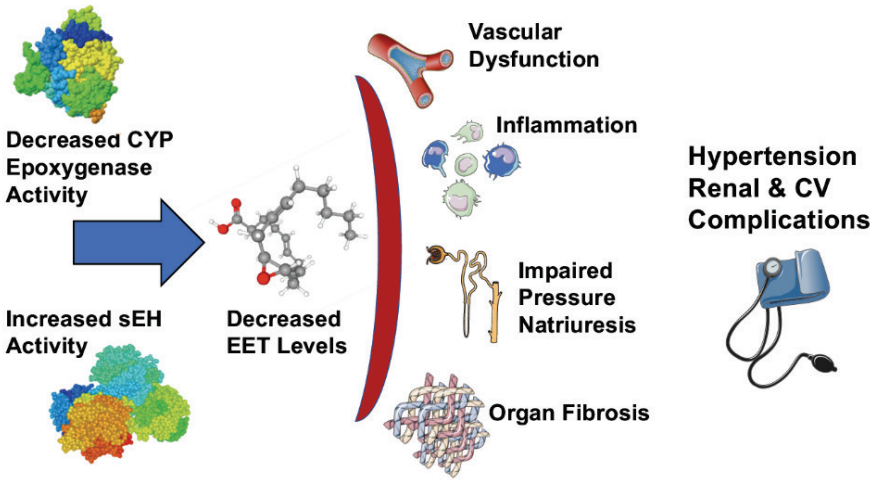

Fig. 2. Decreased epoxyeicosatrienoic acid (EET) levels result in hyper-tension and renal and cardiovascular (CV) complications. Left panel illustrates decreased CYP epoxygenase or increased soluble epoxide hydrolase (sEH) activity leads to decreased EET levels. Right panel illustrates vascular dysfunction, inflammation, impaired pressure natriuresis, and organ fibrosis because of decreased EET levels. These changes in vascular function, inflammation, kidney function, and fibrosis contribute to hypertension and renal and CV disease progression.

Anti-inflammatory actions of epoxyeicosanoids

Inflammation is a key contributor to hypertension, cardiovascular diseases, and kidney diseases (Harrison *et al.* 2011, Viel *et al.* 2010). Vascular and kidney inflammation have been implicated in several hypertension animal models (Harrison *et al.* 2011, Imig and Ryan 2013, Viel *et al.* 2010). Elevations in kidney T cells and cytokines like tumor necrosis factor-α (TNF-α) were reported to contribute to angiotensin-dependent hypertension and Dahl salt-sensitive rat hypertension (Harrison *et al.* 2011, Rudemiller *et al.* 2014, Zhang *et al.* 2014). Decreased EET levels and increased sEH activity have been demonstrated to contribute to vascular and kidney inflammation in hypertension (Fig. 2) (Manhiani *et al.* 2009, Node *et al.* 1999, Zhao *et al.* 2004). On the other hand, increasing EET levels or decreased sEH activity have been demonstrated to decrease inflammation and blood pressure in animal models of hypertension (Deng *et al.* 2011, Manhiani *et al.* 2009, Olearczyk *et al.* 2009). EETs have been demonstrated to decrease kidney macrophage infiltration in hypertension (Olearczyk *et al.* 2009, Zhao *et al.* 2004). Likewise, increasing EET levels in the kidney decrease interleukin-6 (IL-6), TNF-α, and monocyte chemoattractant protein-1 (MCP-1) levels in animal models of hypertension (Campbell *et al.* 2017, Olearczyk *et al.* 2009, Zhao *et al.* 2004). These anti-inflammatory EET actions are mediated by downregulation of the transforming growth factor-β1 (TGF-β1)/Smad3 signaling, inhibition of IKK activity, and nuclear factor-κB activation (Imig 2012, Manhiani *et al.* 2009, Node *et al.* 1999). These findings indicate that sEH inhibition or increasing EET levels can decrease vascular and kidney inflammation to lower blood pressure in hypertension.

Increasing epoxyeicosanoids lowers blood pressure and improves renal and cardiovascular outcomes in hypertension

There have been two pharmacological approaches developed to manipulate EETs to lower blood pressure and combat cardiovascular morbidity and mortality in hypertension. The cardiovascular and renal actions of these pharmacological approaches agree with genetic approaches to increase EET levels (Campbell *et al.* 2017, Imig 2018, Imig and Hammock 2009, Yang *et al.* 2017). Antihypertensive actions were first demonstrated with sEH enzyme-based drugs in spontaneously hypertensive rats (SHR) and angiotensin-dependent hypertension (Imig *et al.* 2002, Yu *et al.* 2000). Several years later EET analogs that mimic the actions of endogenous EETs were demonstrated to lower blood pressure in SHR and angiotensin-dependent hypertension (Imig *et al.* 2010, Khan *et al.* 2014). The positive antihypertensive actions for sEH inhibitors and EET analogs have been attributed to anti-inflammation, vasodilation, and natriuresis (Fig. 3) (Campbell *et al.* 2017, Capdevila and Wang 2013, Imig 2012, Imig 2018, Imig and Hammock 2009). Details on the development of sEH inhibitors and EET analogs can be found in several exceptional review articles (Campbell *et al.* 2017, Capdevila and Wang 2013, Imig 2018, Imig and Hammock 2009, Marino 2009).

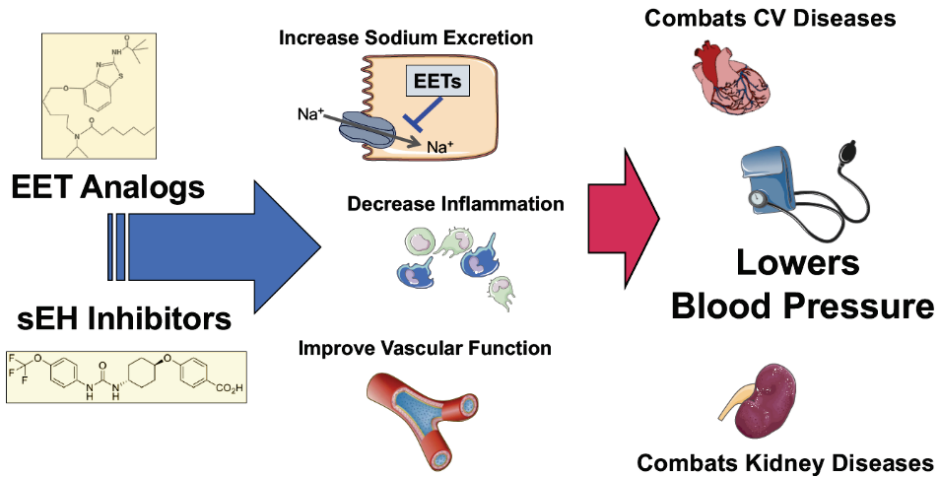

Fig. 3. Epoxyeicosatrienoic acid (EET) analogs and soluble epoxide hydrolase (sEH) inhibitors lowers blood pressure and combats cardiovascular (CV) and renal diseases. Left to right: EET analogs or sEH inhibitors increase sodium excretion, decrease inflammation, and improve vascular function to lower blood pressure and combat CV and renal diseases.

Rapid development of sEH inhibitors resulted in the first human clinical trial and these sEH inhibitors continue to be tested in humans for beneficial cardiovascular actions (Imig and Hammock 2009). The positive kidney, heart, and vascular actions attributed to sEH inhibitors are associated with an increase in EET levels (Imig 2018, Imig and Hammock 2009, Marino 2009). A major advantage for sEH inhibitors was the early development of orally active inhibitors that allowed for chronic animal studies (Imig and Hammock 2009).

Experimental studies in animal models determined that sEH inhibitors or sEH (EPHX2) gene deletion were antihypertensive with vasodilatory and natriuretic actions (Imig *et al*. 2002, Manhiani *et al.* 2009, Yu *et al*. 2000, Zhao *et al.* 2003). Additional studies revealed that sEH inhibitors have anti-inflammatory and anti-fibrotic actions that decreases kidney and cardiac damage that occurs in hypertension (Imig 2018, Imig and Hammock 2009). Increasing epoxyeicosanoid levels *via* sEH inhibition improves the pressure-natriuretic relationship and attenuates angiotensin-dependent hypertension (Honetschlägerová *et al.* 2011a, Honetschlägerová *et al.* 2011b, Imig *et al*. 2002, Zhao *et al.* 2003). The blood pressure lowering action demonstrated by sEH inhibition in rats with angiotensin II malignant hypertension was due to an increase in renal EET levels and interactions with nitric oxide renal hemodynamic and sodium excretion actions (Honetschlägerová *et al.* 2013, Sporková *et al*. 2014, Varcabová *et al*. 2013). These studies revealed an interaction between sEH inhibition, EETs, and the renin-angiotensin system that contributes to the beneficial actions in hypertension (Sporková *et al*. 2014, Varcabová *et al*. 2013). Beneficial actions beyond lowering blood pressure have been observed with sEH inhibitors and EPHX2 gene deletion. Deoxycorticosterone acetate high salt (DOCA-salt) hypertension has a lower blood pressure in EPHX2 gene-deleted mice (Manhiani *et al*. 2009). In addition to blood pressure lowering in DOCA-salt hypertension, EPHX2 gene deletion attenuated kidney inflammation and glomerular injury (Manhiani *et al*. 2009). Inhibition of soluble epoxide hydrolase slows the progression of cardiac hypertrophy and renal tubulointerstitial and glomerular injury in 5/6 nephrectomized Ren-2 transgenic hypertensive rats (Kujal *et al*. 2014). These findings in kidney and cardiovascular disease animal models led to a rapid development for sEH inhibitors towards human clinical trials.

Human studies with sEH inhibitors have been variable in terms of ability to combat cardiovascular diseases (Imig and Hammock 2009, Yang *et al*. 2017). Initial clinical trials with sEH inhibitors evaluated type 2 diabetes and hypertension (Imig and Hammock 2009). Although efficacy was not established in the Phase II clinical trial, the sEH inhibitor was found to be safe to use and to inhibit sEH activity (Imig and Hammock 2009). More promising cardiovascular data were found in the second human clinical trial with sEH inhibitors (Yang *et al*. 2017). Forearm blood flow responses in obese humans with COPD indicated that endothelial dependent blood flow responses were improved (Yang *et al*. 2017). Several sEH inhibitor clinical trials are in various stages and include evaluating their therapeutic effects in subarachnoid hemorrhage (Imig 2018). Recent human studies also support the potential benefit for using sEH inhibitors to prevent or treat acute kidney injury following cardiac or thoracic surgeries (Shuey *et al.* 2017). Collectively, these findings indicate that sEH inhibitors are still a potential therapeutic for hypertension, kidney diseases, and cardiovascular diseases.

The development and testing of EET analogs has been slower due to hurdles in advancing to orally bioavailable EET analogs, multiple EETs with varied biological actions, and the lack of defined protein target (Campbell *et al.* 2017, Falck *et al.* 2014, Sudhahar *et al.* 2010). EET analog development has progressed based on structure activity relationship studies conducted in mesenteric resistance arteries, coronary arteries, and renal afferent arterioles (Campbell *et al.* 2017, Sudhahar *et al.* 2010). These studies led to 11,12-EET and 14,15-EET agonists and antagonists (Campbell *et al.* 2017, Falck *et al.* 2014). Subsequent modifications to the EET agonists resulted in orally bioavailable EET analogs (Campbell *et al.* 2017, Falck *et al.* 2014). These oral EET analogs were then tested in several hypertension and renal and cardiovascular disease animal models (Campbell *et al.* 2017).

Antihypertensive actions of EET analogs and genetic CYP2C expoxygenase overexpression were demonstrated in rat and mice hypertension models (Imig *et al.* 2010, Khan *et al.* 2014, Lee *et al.* 2010). EET analogs demonstrated an ability to lower blood pressure in SHR, angiotensin-dependent hypertension, and salt-sensitive hypertension (Campbell *et al.* 2017, Imig 2018). The decrease of blood pressure in response to

EET analogs can be attributed to vasodilation and blocking epithelial sodium reabsorption (Khan *et al.* 2014). EET analogs attenuate the development of angiotensin-dependent malignant hypertension through suppression of angiotensin vasoconstriction and sodium reabsorption (Jíchová *et al.* 2016). On the other hand, EET analogs failed to lower blood pressure or attenuate end-organ damage when administered during the late malignant hypertension phase (Jíchová *et al.* 2016). Overexpressing epoxygenases in mouse endothelial cells results in increased endothelial EET synthesis, decreased blood pressure and decreased glomerular injury in angiotensin high salt hypertension (Lee *et al*. 2010). Cardiac beneficial actions for EET analogs have been determined in ischemic cardiac disease and hypertension-induced cardiac hypertrophy (Batchu *et al*. 2011, Khan *et al*. 2013, Neckář *et al*. 2018). The cardiac protective actions for EET analogs is due to vasodilatory, anti-fibrotic, and anti-inflammatory actions (Batchu *et al*. 2011, Khan *et al*. 2013, Neckář *et al*. 2018). Kidney protective actions have been determined in several acute and chronic kidney disease animal models (Campbell *et al.* 2017, Imig 2018). The EET analog, EET-A, lowered blood pressure, decreased the CXC chemotaxis inflammatory axis, and decreased renal fibrosis in female lupus nephritis mice (Khan *et al*. 2019). Overall, EET analogs have been widely demonstrated to decrease kidney disease progression in diabetes, hypertension, drug-induced nephrotoxicity, and radiation-induced kidney injury (Campbell *et al.* 2017, Imig 2018). The beneficial effects of EET analogs are a combination of improving endothelial function, decreasing renal inflammation, reducing apoptosis, and opposing epithelial to mesenchymal mediated fibrosis (Campbell *et al.* 2017, Imig 2018). Currently, EET analogs are moving towards clinical trials for kidney and cardiovascular diseases.

Conclusions

Epoxyeicosanoids have been extensively studied in hypertension and kidney and cardiovascular diseases associated with hypertension. These experimental studies in animal models and humans have demonstrated that decreased EET levels or increased sEH activity contributes to compromised endothelial and vascular function and epithelial sodium transport in hypertension (Campbell *et al.* 2017, Imig 2015, Imig and Hammock 2009). Cardiovascular mortality and kidney damage associated with hypertension can also be attributed to decreases in epoxyeicosanoids (Campbell *et al.* 2017, Imig and Hammock 2009). Inflammation resulting from decreased EET levels contributes to these detrimental cardiovascular and kidney hypertension complications (Campbell *et al.* 2017, Imig and Hammock 2009). Pharmacological and genetic manipulation of sEH, epoxygenase enzymes, and epoxyeicosanoid levels have determined that EETs dilate arterioles, decrease renal tubular sodium reabsorption, decrease fibrosis, and decrease inflammation (Campbell *et al.* 2017, Imig 2015, Imig and Hammock 2009). These epoxyeicosanoid actions not only contribute to blood pressure lowering in hypertension but also combat renal and cardiovascular disease (Campbell *et al.* 2017, Imig 2015, Imig and Hammock 2009). Likewise, the pharmacological development of sEH inhibitors and EET analogs demonstrates great potential including studies in humans to treat hypertension and associated complications (Campbell *et al.* 2017, Imig 2018). A recent study provides evidence that combining sEH inhibitors with EET analogs does not provide additive antihypertensive or cardioprotective effects in angiotensin-dependent hypertension (Červenka *et al.* 2018). This finding supports the notion that sEH inhibitors and EET analogs have overlapping actions to lower blood pressure and decrease renal and cardiovascular disease progression (Červenka *et al.* 2018). In conclusion, there is a bright future for sEH inhibitors or EET analogs as a treatment for hypertension and to combat cardiovascular mortality and kidney disease.

Conflict of Interest

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

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