Cytochrome P450 eicosanoids in hypertension and renal disease

Fan Fan, Yoshikazu Muroya, and Richard J. Roman

Purpose of review
Cytochrome (CYP) P450 metabolites of arachidonic acid, 20-hydroxyeicosatetraenoic acid (20-HETE) and epoxyeicosatrienoic acids (EETs) contribute to the regulation of renal tubular and vascular function. This review highlights the results of the recent genetic studies in humans and rodent models, indicating that these eicosanoids participate in the control of blood pressure (BP), chronic kidney disease (CKD), renal ischemia–reperfusion injury (IRI) and polycystic kidney disease (PKD).

Recent findings
Endogenous 20-HETE has been reported to play an essential role in the myogenic and tubuloglomerular feedback responses in the afferent arteriole, and a deficiency of 20-HETE contributes to the development of hypertension and renal injury in Dahl S rats. Mutations in CYP4A11 and CYP4F2 have been linked to elevated BP in humans. EETs have been shown to regulate epithelial sodium channel in the collecting duct, lower BP and have renoprotective properties. 20-HETE also opposes the development of CKD and IRI, and may play a role in PKD.

Summary
These studies indicate that CYP P450 metabolites of arachidonic acid play an important role in the control of BP, CKD, AKI and PKD. Drugs targeting these pathways could be useful in the treatment of IRI and CKD.

Keywords
acute kidney injury, chronic kidney disease, epoxyeicosatrienoic acids, 20-HETE, hypertension and diabetic nephropathy, polycystic kidney disease

INTRODUCTION
Previous studies have revealed that arachidonic acid is metabolized by the cytochrome P450 (CYP) enzymes of the 4A and 4F families to 20-hydroxyeicosatetraenoic acid (20-HETE) and by CYP2C and CYP2J pathways to epoxyeicosatrienoic acids (EETs), and that these compounds influence both renal tubular transport and renal and peripheral vascular tone. 20-HETE is a potent vasoconstrictor and inhibits sodium transport in the proximal tubule and thick ascending loop of Henle (TALH), whereas EETs are endothelium-derived relaxing factors that dilate small arterioles and inhibit sodium transport in the proximal tubule and the collecting duct [1].

The renal formation of 20-HETE and EETs is altered in the various models of hypertension, and drugs that target these pathways have been reported to alter the development of hypertension and renal injury in the preclinical studies. In the last few years, mutations in CYP4A11, CYP4F2 that produce 20-HETE, soluble epoxide hydrolase (sEH) that inactivate EETs and more recently uridine 5'-diphosphoglucuronosyltransferase (UDP-glucuronosyl transferase, UGT) involved in the inactivation and elimination of 20-HETE have been linked to variation in the blood pressure (BP) in a variety of human population studies and in rodent models [2,3,*4,5,*6,7,*]. 20-HETE and EETs have renoprotective actions in hypertension, chronic kidney disease (CKD) and renal ischemia–reperfusion injury (IRI) [1,5,*6,7,*8,*9,*10–12]. More recent studies have indicated that EETs regulate the activity of the epithelial sodium.


KEY POINTS

- Sequence variants in CYP4A11 and CYP4F2 are linked to the development of hypertension in numerous patient populations.
- 20-HETE plays an essential role in the myogenic and TGF responses in the Af-art.
- A deficiency in the formation of 20-HETE and EETs is associated with the development of salt-sensitive hypertension.
- EETs reduce inflammation and are renoprotective.
- 20-HETE may play roles in AKI and PKD.

channel (ENaC) in the collecting duct [8∗∗], and that 20-HETE is pro-proliferative and may participate in the pathogenesis of polycystic kidney disease (PKD) [13∗∗,14–16]. This review highlights the emerging evidence that metabolites of arachidonic acid play important roles in the control of BP, diabetic nephropathy, acute kidney injury (AKI) and PKD, and discusses the results of the recent studies using newly developed knockout and transgenic mouse and rat models that explore the mechanisms involved.

EFFECTS OF 20-HYDROXYEICOSATETRAENOIC ACID ON THE RENAL VASCULAR TONE

Previous studies suggested that 20-HETE plays an important role in the regulation of vascular tone [1]. 20-HETE is a potent vasoconstrictor (EC_{50} < 10^{-8} M) that is produced by the renal and cerebral arteries. It activates protein kinase C (PKC), mitogen-activated protein kinases (MAPKs), tyrosine kinase and Rho kinase pathways to promote Ca^{2+} entry through depolarization of vascular smooth muscle cells (VSMCs) secondary to the blockade of the large conductance calcium-sensitive K^{+} (BK) channel [1,17,18]. 20-HETE also increases the conductance of L-type Ca^{2+} channels through activation of PKC [19] and enhances inward nonselective cation currents through transient receptor potential canonical 6 (TRPC6) channels [20], which are implicated in the myogenic response [21]. Previous reports also have indicated that elevations in transmural pressure increases the production of 20-HETE [22], and that inhibition of the synthesis of 20-HETE impairs the development of active myogenic tone in the renal and cerebral arteries [1,18].

Inhibitors of the synthesis of 20-HETE have been reported to impair the autoregulation of renal and cerebral blood flow (RBF and CBF) in vivo [1,18]. Analysis of the time course of the impaired RBF autoregulatory response to elevations in systemic pressure after blockade of the formation of 20-HETE indicates that 20-HETE primarily affects the rapid myogenic component of RBF autoregulation more than tubuloglomerular feedback (TGF) in vivo. However, direct evidence that endogenously formed 20-HETE plays a role in the myogenic and TGF responses at the level of the afferent arteriole (Af-art) was lacking. Our laboratory recently reported that administration of an inhibitor of the synthesis of 20-HETE or a 20-HETE antagonist completely blocks the myogenic response of the Af-art to elevations in the transmural pressure [23∗∗]. These effects were reversed by the exogenous administration of a 20-HETE mimetic [1]. We also found that 20-HETE plays a key role in the TGF-mediated regulation of the Af-art using the double-perfused rabbit Af-art/macula densa preparation [23∗∗].

Upregulation of the formation of 20-HETE in the renal microcirculation will enhance Af-art vascular resistance, leading to reductions in glomerular capillary pressure (Pgc) and glomerular filtration rate (GFR). It also lowers the pressure in the postglomerular circulation that shifts the pressure natriuresis relationship to higher pressures [24] and promotes the development of hypertension. However, 20-HETE-mediated elevations in renal vascular resistance also reduce the transmission of pressure to the glomerular circulation and oppose the development of hypertension-induced glomerular injury. This view is consistent with the previous results in the spontaneously hypertensive rat (SHR) which has elevated production of 20-HETE and pregglomerular vascular resistance, and is protected from hypertension-induced renal end-organ damage [1,18].

On the other hand, the formation of 20-HETE has recently been reported to be reduced in the pregglomerular vasculature of Dahl salt sensitive rats (Dahl S) [25∗∗,26∗∗]. These rats exhibit impaired myogenic and TGF responses of Af-art [26∗∗], autoregulation of RBF, elevated Pgc, and rapidly develop proteinuria and glomerular injury following the onset of hypertension [26∗∗,27]. Our data also indicated that transfer of the CYP4A genes that are responsible for the formation of 20-HETE from Brown Norway (BN) rats to Dahl S genetic background in a chromosome 5 consomic strain [26∗∗] and in a newly developed CYP4A1 sleeping beauty transposon transgenic Dahl S rat [28] restored the production of 20-HETE and the myogenic response of the Af-art, and protected from the development of hypertension-induced renal injury [27]. The formation of 20-HETE in the renal circulation is also reduced in both type I and type II diabetic animal models that present with hyperfiltration and...
develop glomerular disease [29,30]. Overall, these findings suggest that genetic and dietary-induced modulation of the expression of CYP4A enzymes in the renal microcirculation alters the Af-art tone and the susceptibility to develop renal end-organ damage.

**EFFECTS OF EPOXYEICOSATRIENOIC ACIDS ON THE RENAL VASCULAR TONE**

EETs are formed by the enzymes of the CYP2C and CYP2J families in the proximal tubule, collecting duct and renal vascular endothelium [6,31**]. They act as endothelium-dependent hyperpolarizing factors (EDHFs) in the renal microcirculation by activating the BK channel in VSMCs and are hydrolyzed by sEH in vivo to less biologically active dihydroxyeicosatrienoic acids (DHETs). EETs contribute to the nitric oxide and cyclooxygenase (COX) independent components of the vasodilator response of the Af-art to acetylcholine (Ach), bradykinin and adenosine [31**]. Recent studies have revealed that EETs activate cell-surface receptors to increase the levels of cyclic adenosine monophosphate (cAMP) that activates the BK channel, resulting in vasodilation. The vasodilatory effect of EETs is also partially due to stimulation of protein phosphatase 2A (PP2A) and activation of the BK channel in preglomerular arterioles [31**]. There is now evidence for a role for transient receptor potential cation channel, subfamily V, member 6 (TRPV6) channels in the activation of the BK channel following administration of EETs. EETs also activate small and intermediate calcium-activated potassium (KCa) channels in the endothelium that alters the driving force for calcium entry, and possibly the generation and release of nitric oxide [31**].

Much of the recent work in this area has focused on the development of EETs antagonists and stable agonists [31**,32,33**]. 14,15 Epoxycosa-5(Z)-enoic acid (14,15 EEZE) has been characterized as an antagonist that inhibits the response to all four regioisomers of EETs, whereas 14,15 epoxycosa-5(Z)-enoic-methylsulfonylimide (14, 15 EEZE-SI) is a more selective inhibitor of the vasodilator response to 14,15 EET. Administration of these compounds blunts the vasodilator responses to Ach and bradykinin [31**].

Upregulation of the formation of EETs in the endothelium of transgenic mice expressing human CYP2J2 or CYP2C8 epoxygenases enhances the vasodilator response of the Af-art to Ach and attenuates the response to endothelin. These animals also exhibit less hypertension in response to the chronic blockade of nitric oxide or infusion of angiotensin II (ANG II) [34]. Similarly, Sun et al. [5**] reported that the vasodilator responses to Ach and bradykinin were potentiated in sEH knockout mice, in which the levels of EETs are elevated [35,36].

**20-HYDROXYEICOSATETRAENOIC ACID AND EPOXYEICOSATRIENOIC ACIDS IN THE REGULATION OF SODIUM TRANSPORT**

In addition to altering the vascular tone, 20-HETE inhibits sodium-hydrogen exchanger 3 (NHE3)-mediated sodium and water reabsorption in the proximal tubule and sodium-potassium-chloride (NKCC2) transporter in the TALH [1]. Elevations in renal perfusion pressure increase the levels of 20-HETE in the kidney and it participates in the pressure natriuretic response [24]. 20-HETE inhibits sodium transport in the proximal tubule by inhibiting Na⁺–K⁺-ATPase activity by stimulating PKC to phosphorylate the α-subunit, and it also causes internalization of NHE3 [37]. Other studies have shown that the inhibitory effects of parathyroid (PTH), dopamine, endothelin, nitric oxide and ANG II on Na⁺–K⁺-ATPase activity and Na⁺ transport in the proximal tubule are dependent on the formation of 20-HETE [1,18]. In this regard, Fernandez et al. [38] recently reported that 20-HETE inhibitors reduced the natriuretic response to dopamine by 65%. 20-HETE is also produced in the TALH and inhibits the NKCC2 transporter. This is due in part to an ouabain-like effect of 20-HETE to inhibit Na⁺–K⁺-ATPase activity. 20-HETE also blocks a 70 pS K⁺ channel in the apical membrane of the TALH cells [39,40] that limits K⁺ availability for transport via the NKCC2 transporter and reduces the luminal-positive transepithelial potential that serves as the driving force for the passive reabsorption of Na⁺ in the TALH [1]. Elevated levels of 20-HETE have been reported to mediate the inhibitory effects of ANG II, bradykinin and calcium on sodium transport in this portion of the nephron [1]. The inhibitory effects of nitric oxide and carbon monoxide on K⁺ channel activity and Na⁺ transport in the TALH also appear to be dependent on the blockade of the formation of 20-HETE [1,7**,18].

EETs are formed in the proximal tubule and cortical collecting duct, and inhibit sodium transport at both sites. EETs have previously been reported to serve as second messengers for the natriuretic actions of ANG II in the proximal tubule by inhibiting the Na⁺/H⁺ exchanger [1,7**]. More recently, Zhang et al. [41**] reported that the expression of renal CYP2C44 and the formation of EETs in mice are stimulated by dopamine, and that elevation of dopamine level with gludopa increased urine volume and sodium
excretion in wildtype mice but not in CYP2C44 knockout mice.

Most of the recent advances in this field have focused on the role of EETs in the regulation of ENaC and sodium transport in the cortical collecting duct (CCD). Wei et al. [42] previously reported that arachidonic acid inhibited ENaC activity and that this effect was blocked by an epoxygenase inhibitor. Administration of EETs mimicked the effect of arachidonic acid. The expression of CYP2C23 and CYP2C44 epoxygenases are upregulated in the kidney of rats and mice fed high sodium or potassium diets, and blockade of the formation of EETs have been reported to promote sodium retention and raise BP in rats [7**]. More recently, Sun et al. [43] reported that the expression of CYP2C44 and the formation of EETs are enhanced in mice fed a high-potassium diet but not in CYP2C44 knockout mice. Upregulation of the formation of EETs appears to be essential for suppressing the ENaC activity to maintain sodium balance in mice fed a high-potassium diet. CYP2C44 knockout mice exhibit sodium retention and BP increases when they are fed a high potassium diet. The mechanism by which EETs regulate ENaC activity was further explored by Pavlov et al. [44] using patch clamp techniques. They found that EETs rapidly decrease the open-channel probability of ENaC channels, probably via a cAMP/protein kinase A (PKA)-dependent mechanism as well as through redistribution of the channels from the plasma membrane. More recently, Wang et al. [8**] has also reported that a high-salt diet increases the expression of CYP2C44 in the TALH. The significance of this finding, however, remains to be determined as the effects of EETs on sodium transport in the TALH has not been studied.

ROLE OF CYTOCHROME EICOSANOIDS IN HYPERTENSION

The role of 20-HETE in the development of hypertension remains unsettled because 20-HETE has both prohypertensive and antihypertensive properties. It not only increases renal and peripheral vascular tone, but also inhibits sodium transport. In contrast, EETs are vasodilators and natriuretic substances that lower BP. The original studies by Iwai and McGiff et al. indicated that the expression of CYP4A2 and the production of 20-HETE are elevated in SHR, and that blockade of the formation of 20-HETE lowers BP in this model [1]. Similarly, 20-HETE levels are increased by ANG II, and 20-HETE inhibitors attenuate the vasoconstrictor and hypertensive response to ANG II [1,17].

More recent studies have focused on the role of 20-HETE in androgen-dependent models of hypertension. Androgens increase the expression of CYP4A8 and CYP4A12 in rats and mice, respectively [7**,31**]. Administration of dihydrotestosterone (DHT) increases the arterial pressure, and this is associated with the induction of vascular CYP4A expression and increased formation of 20-HETE, oxidative stress and endothelial dysfunction [45]. Treatment with inhibitors of the synthesis or actions of 20-HETE attenuates the rise in arterial pressure [45]. Postmenopausal hypertension is another condition associated with the elevated levels of androgens. Yanes et al. [46] recently reported that elevated vascular production of 20-HETE contributes to the elevation in BP seen in aging female rats. Male CYP4A14 knockout mice also develop an androgen-dependent hypertension that is associated with an increase in the expression of CYP4A12, and the production of 20-HETE in both the kidney and the vasculature [7**,47,48]. In addition, Wu et al. [49**] have now reported that upregulation of the expression of CYP4A12 in a conditional transgenic mouse model produces a 20-HETE-dependent hypertension. The hypertension in both models is rapidly reversed by the administration of a 20-HETE antagonist [49**]. Others have reported that overexpression of human CYP4F2 in a transgenic mouse model is associated with an increase in the renal formation of 20-HETE and arterial pressure [49**,50]. However, the mechanism of the hypertension in all of these models remain to be determined because 20-HETE levels are elevated in both kidney and vasculature, and the hypertension that develops is not salt sensitive. In contrast to the view that elevated levels of 20-HETE promotes hypertension, the expression of CYP4A enzymes and the levels of 20-HETE are reduced in Dahl S rats. They rapidly develop salt-sensitive hypertension that is associated with impaired pressure natriuretic response and enhanced sodium transport in the TALH [18,27]. Upregulation of the formation of 20-HETE with fbrates or transfer of wildtype CYP4A alleles from normotensive strains attenuate the development of hypertension in these animals [18]. Dahl S rats also fail to upregulate epoxygenase activity when fed a high-salt diet, and a deficiency in the formation of EETs has been suggested to also contribute to the development of hypertension in this strain [51].

In humans, a T-to-C substitution at nucleotide 8590 in the CYP4A11 isoform that decreases the formation of 20-HETE has been linked to the development of hypertension [52–54]. This same polymorphism in CYP4A11 is also associated with the incidence of hypertension in a cohort of patients with myocardial infarction in Germany [55,56]. Moreover, a V433M variant in the CYP4F2 that also
CYP4A11 decreases the formation of 20-HETE [57] has been linked to the incidence of hypertension, cardiovascular disease [58–60] and stroke in several human population studies [61,62]. A different G421C single nucleotide polymorphism (SNP) in the CYP4F2 is also associated with elevated BP in a Chinese population [60,63]. Finally, an Australian group identified a G-to-A polymorphism in CYP4F2 that reduces 20-HETE production and is also associated with hypertension [58].

The association studies linking sequence variants in CYP4A11 and CYP4F2 genes to hypertension are consistent with the previous findings in Dahl S rats, suggesting that a deficiency in the formation of 20-HETE promotes salt-sensitive hypertension. However, the mechanism involved remains to be determined because the renal excretion of 20-HETE has been reported to be increased rather than decreased in hypertensive patients carrying the mutant CYP4F2 allele [58,60]. These authors concluded that elevated 20-HETE levels might promote hypertension by causing renal vasoconstriction. However, there is no evidence that RBF or GFR is altered in hypertensive patients with elevated urinary 20-HETE levels. Another concern is that 20-HETE is excreted as a glucuronide conjugate and not in the free form. In this regard, Driesbach et al. [64*] recently reported that the levels of 20-HETE glucuronide in the plasma of patients are greater than that found in urine and that the fractional excretion is less than 1%. Thus, urinary 20-HETE is not of renal origin and simply reflects circulating levels that are filtered and excreted.

EETs promote vasodilation, increase sodium excretion and lower arterial pressure [7**,31**]. Previous studies have reported that administration of an epoxygenase inhibitor increases BP in rats fed a high-salt diet [7**]. Renal epoxygenase activity increases in salt-resistant rats fed a high-salt diet but not in Dahl S rats that develop hypertension [51]. CYP2C44 knockout mice also develop hypertension when fed high salt [65**]. This is associated with an increase in the activity of the ENaC because of reductions in extracellular-signal-regulated kinases 1/2 and ENaC subunit phosphorylation, and the hypertension can be prevented by the administration of amiloride, an inhibitor of ENaC [50]. CYP2C44 knockout mice also develop hypertension when fed a high-potassium diet, indicating that upregulation of the formation of EETs is required to prevent sodium retention in animals when aldosterone levels are increased [8**]. Additional evidence of a role for reduced epoxygenase activity was obtained in the studies of a CYP4A10 knockout mouse. Although this isoform does not metabolize arachidonic acid to 20-HETE, disruption of this gene produced salt-sensitive hypertension [66]. The hypertensive phenotype was associated with a reduction in the renal synthesis of EETs and enhanced ENaC activity in the CCD [66]. Treatment with amiloride normalizes the BP in the CYP4A10 knockout mouse [66]. Other investigators have shown that administration of inhibitors of sEH to increase renal production of EETs or stable EETs analogs lowers the BP in the experimental animal models of hypertension [31**,67].

CYP2C8, CYP2C9 and CYP2J2 are the primary epoxygenases that produce EETs in humans, and sEH metabolizes EETs to less active products. Several variants of these genes have been identified that have different catalytic activity. However, studies in both Caucasian and African-American populations have failed to identify the associations with these genes and hypertension [2,7**]. There is evidence of an association with CYP2C9 variant and hypertension in a subset of Chinese women [68]. Mixed results have been reported for a variant in CYP2J2 and hypertension in different populations. Several polymorphisms in sEH have been identified, but none has been linked to hypertension [7**,31**].

**ROLE OF CYTOCHROME EICOSANOIDS IN HYPERTENSIVE AND DIABETIC NEPHROPATHY**

Increased transmission of pressure to the glomerular capillaries has long been thought to play a key role in the development of glomerulosclerosis. The recent findings that a deficiency in the formation of 20-HETE impairs both myogenic and TGF-β responses of Af-α in Dahl S rats [25**,26**] is consistent with the rise in Pgc, upregulation of the renal expression of transforming growth factor beta (TGF-β), increased permeability of the glomerulus to albumin (Palb), and the rapid development of proteinuria and renal injury seen in Dahl S rats [18,27]. Consistent with a glomerular protective action of 20-HETE, Driesbach et al. [64**] reported that the renal excretion of 20-HETE is negatively correlated with the progression of CKD in African-American patients. The formation of 20-HETE in the renal circulation has been reported to be reduced in a streptozotocin (STZ)-treated type I and fat-fed type II [29,30] diabetic rats, but it remains to be determined whether this contributes to the development of hyperfiltration or glomerular disease in these models. In contrast, renal vascular 20-HETE levels and reactivity are elevated in SHR, ANG II and androgen dependent models of hypertension that are protected from the development of proteinuria and renal injury [1,27].
There is also evidence that 20-HETE has a direct renoprotective effect on the glomerular filtration barrier [24]. 20-HETE is produced in the glomerulus and in cultured podocytes. TGF-β directly increases PAlb in isolated glomeruli and inhibits the formation of 20-HETE. Moreover, administration of 20-HETE prevents the effects of TGF-β on PAlb [69], and blockade of the formation of 20-HETE increases PAlb and proteinuria in the normal rats [70]. The formation of 20-HETE in the glomerulus is also reduced in type I and II diabetic rats, and upregulation of the formation of 20-HETE with fibrates affords renoprotection [30]. These findings indicate that 20-HETE produced in the glomerulus plays a critical role in maintaining a glomerular filtration barrier to albumin, and that upregulation of TGF-β in hypertension may initiate the development of proteinuria and renal disease in part by inhibiting the formation of 20-HETE.

On the other hand, Eid et al. [71,72] recently reported that high glucose increases the expression of CYP4A and the production of 20-HETE, at least transiently, in the cultured podocytes and renal epithelial cells. This was associated with an increase in the production of reactive oxygen species and apoptosis. These effects were blocked with an inhibitor of the synthesis of 20-HETE. Inhibition of the formation of 20-HETE is also effective at reducing oxidative stress, tubular injury and extracellular matrix formation in cultured rat renal proximal tubular cells [9\*]. In type I diabetic OVE26 mice, upregulated expression of CYP4A and Nox1 oxidase in the glomerulus increases oxidative stress that contributes to the loss of podocytes apoptosis and subsequent proteinuria [72].

EETs have been found to be renoprotective in the experimental animal models of hypertension and diabetes [31\*\*]. Chronic administration of sEH inhibitor protects against the development of proteinuria and glomerular injury in ANG II and deoxycorticosterone acetate (DOCA)-salt hypertensive rats and mice [31\*]. sEH knockout mice had lowered BP, renal inflammation and glomerular injury following induction of hypertension with DOCA-salt or ANG II [31\*\*] or diabetes with STZ. More recently, chronic administration of an EET analog has been reported to reduce BP and renal injury in SHR and ANG II hypertensive mice [33\*\*]. The production of EETs is reduced in the kidney and glomeruli [9\*,10,30,73] of STZ-treated animals and in the glomeruli exposed to high glucose. Upregulation of the expression of EETs in CYP2J2 transgenic mice also reduces proteinuria, renal inflammation and glomerular injury following induction of diabetes with STZ [73].

**ROLE OF CYTOCHROME EICOSANOIDS IN ACUTE KIDNEY INJURY**

There are three reports that have addressed the role of 20-HETE on renal IRI. Regner et al. [74] reported that a 20-HETE mimetic protects the kidney from the injury induced by bilateral renal IRI by preventing the delayed postischemic fall in medullary blood flow that occurs about 2 h after reperfusion [74]. Another report by Hoff et al. [75] showed that pretreatment of the animal with a 20-HETE inhibitor (HET0016) or a 20-HETE antagonist (6, 15–20-HEDE) protected against renal ischemia injury in a uninephrectomized model designed to mimic renal transplant injury. The reason for the differences in the results of the studies seems to be related to the model systems, bilateral versus unilateral ischemia, because in a follow-up study, Roman et al. [76] confirmed that inhibition of the formation of 20-HETE is protective in the unilateral ischemic kidney model but enhances injury in the bilateral ischemia models.

The therapeutic potential of EET antagonists in IRI have yet to be evaluated. There is only one study which indicates that administration of a sEH inhibitor to increase the intrarenal levels of EETs attenuates the rise in serum creatinine concentration and tubular necrosis in mice following IRI [77].

**ROLE OF CYTOCHROME EICOSANOIDS IN POLYCYSTIC KIDNEY DISEASE**

PKD is a genetic disorder of the kidneys characterized by the presence of multiple cysts. Our group reported that the renal production of 20-HETE is elevated in a well characterized model of PKD and that blockade of the formation of 20-HETE with HET0016 reduced cystic disease [15,16]. More recently, Klawitter et al. [13\*] reported that serum 20-HETE levels are elevated in PKD patients, and it is significantly correlated with estimated GFR (eGFR) and total kidney volume. Given that 20-HETE promotes the proliferation of renal epithelial cells and activates PKC, Src and eGFR pathways [14] that are implicated in PKD, these findings suggest that 20-HETE might be a new biomarker and a therapeutic target for PKD.

**DRUG INTERACTIONS WITH CYTOCHROME P450 EICOSANOIDS**

EETs are produced by the CYP2C8, CYP2C9 and CYP2J pathways and inactivated by sEH, whereas 20-HETE is produced by CYP4A and CYP4F pathways and is inactivated by metabolism by β-oxidation, cyclooxygenase 2 (COX2) [77] and conjugation with glucuronic acid via UGT2B7 [78\*]. As the CYP2C8
| Isoform | CYP2C8 | CYP2C9 | CYP2J2 | sHE | CYP4A11 | CYP4F2 | COX2 | UGT2B7/1A9 |
|---------|--------|--------|--------|-----|---------|--------|------|-----------|
| Products | Rifampin, fibrates | Carbamazepine, phenobarbital, phenytoin, rifampin, fibrates | | Clofibrate, naphthoﬂavone, DEHP, 3-methylcholanthrene | Fibrates, androgens, diethyl phthalate, naphthoﬂavone, PPARα | | | |
| Inducers | Gemfibrozil, montelukast, glitazones | Fluconazole, miconazole, ketoconazole, amiodarone, fibrates, sulfaphenazole | Ketoconazole, miconazole, nicardipine, clotrimazol, ivermectin | Valproic acid | Nitric oxide, carbon monoxide, superoxide | Sesamin | Celecoxib, rofecoxib, valdecoxib | |
| Inhibitors | Torsemide, repaglinide, ibuprofen, rosiglitazone, repaglinide, amiodarone | NSAIDs, glyburide, losartan, irbesartan, celecoxib, statins, rosiglitazone, warfarin, tolbutamide | Amiodarone, cyclosporine, aspirin, tamoxifen, terfenadine, vitamin D | Fatty acids | Vitamin K, vitamin E, leukotriene B4 | | | |
| Competitive substrates | | | | | | | | |

**20-HETE**, 20-hydroxyeicosatetraenoic acid; **COX2**, cyclooxygenase 2; **DEHP**, di2-ethylhexyl phthalate; **EETs**, epoxyeicosatrienoic acids; **IL-1β**, interleukin-1 beta; **LPS**, lipopolysaccharides; **PPARα**, peroxisome proliferator-activated receptor alpha; **sEH**, soluble epoxide hydrolase; **TCDD**, 2,3,7,8-tetrachlorodibenzodioxin; **TNFα**, tumor necrosis factor alpha.
and CYP2C9 pathways are the major routes for the metabolism of many commonly prescribed compounds, there is numerous potential for drug interactions that can affect the production of EETs. As summarized in Table 1, the formation of EETs is inhibited by antifungal drugs such as ketoconazole and miconazole. Nonsteroidal anti-inflammatory drugs, sulfonlurea antidiabetic agents, ANG II receptor blockers, statins, calcium channel blockers, troglitazone, warfarin and second-generation antihistamine drugs are all metabolized by CYP2C8 and CYP2C9 families, and potentially act as competitive inhibitors of the formation of EETs [79–81]. On the other hand, fibrates induce the expression of CYP2C9 and downregulate the expression of sEH to increase the formation of EETs. Fibrates, androgens, starvation, α-naphthoflavone and the plasticizer dibutyl phthalate induce the expression of enzymes of the CYP4A pathway and increase the formation of 20-HETE. Physiologically, nitric oxide, carbon monoxide and superoxide bind to the heme-binding site of enzymes of the CYP4A pathway and inhibit the formation of 20-HETE [1]. Numerous drugs such as NSAIDs, opioids, gemfibrozil, furosemide and propanol are substrates for UGT enzymes that could compete for the metabolism of 20-HETE and increase the levels [82]. Finally, Liu et al. [77] reported that inhibitors of COX2 markedly increased the levels of 20-HETE by inhibiting its metabolism by this pathway. These authors further suggested that upregulation of the formation of 20-HETE might contribute to some of the increased cardiovascular risk associated with the administration of inhibitors of COX2.

CONCLUSION

Considerable evidence has accumulated indicating that the synthesis of eicosanoids is altered in the humans and the experimental animal models of hypertension and that these compounds have important effects on renal function and vascular tone and the long-term control of arterial pressure. 20-HETE inhibits Na⁺ transport, and a deficiency in the renal formation of 20-HETE has been linked to the development of hypertension in the Dahl S rats and in hypertensive patients who have inactivating mutations in CYP4A11 and CYP4F2 that produce 20-HETE. Similarly, EETs inhibit ENaC in the collecting duct and loss of renal epoxygenase activity promotes sodium-sensitive hypertension in the knockout mouse models. Both EETs and 20-HETE oppose the development of CKD, and agents that increase the intrarenal levels of EETs and 20-HETE may protect against renal IRI. There is also emerging evidence that 20-HETE may contribute to epithelial cell proliferation and cyst formation in the models of PKD.

Acknowledgements

None.

Financial support and sponsorship

This work was funded in part by the National Institutes of Health grant R01 HL36279 and DK 104184 (to R.J.R.).

Conflicts of interest

None.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

# of special interest

**# of outstanding interest

1. Roman RJ, P-450 metabolites of arachidonic acid in the control of cardiovascular function. Physiol Rev 2002; 82:131–185.
2. Fava C, Ricci M, Melander O, et al. Hypertension, cardiovascular risk and polymorphisms in genes controlling the cytochrome P450 pathway of arachidonic acid: a sex-specific relation? Prostaglandins Other Lipid Mediat 2012; 98:75–85.
3. Ward NC, Tsai IU, Barden A, et al. The effect of a single nucleotide polymorphism of the CYP4F2 gene on blood pressure and 20-hydroxyeicosatetraenoic acid excretion after weight loss. J Hypertens 2014; 32:1498–1502; discussion 1502.
4. Paper showing that CYP4F2 genotype affects blood pressure response to weight loss.

1. Liang JQ, Yan MR, Yang L, et al. Association of a CYP4A11 polymorphism and hypertension in the Mongolian and Han populations of China. Genet Mol Res 2014; 13:508–517.
5. Sun D, Cuevas AJ, Gotlinger K, et al. Soluble epoxide hydrolase-dependent regulation of myogenic response and blood pressure. Am J Physiol Heart Circ Physiol 2014; 306:H1146–H1153.

Evidence that knockout of soluble epoxide hydrolase decreases vascular tone and blood pressure in mice.

6. Kujal P, Certikova Chabova V, Sikaroufkova P, et al. Inhibition of soluble epoxide hydrolase is renoprotective in 5/6 nephrectomized Ren-2 transgenic hypertensive rats. Clin Exp Pharmacol Physiol 2014; 41:227–237.
7. Capdevila J, Wang W. Role of cytochrome P450 epoxygenase in regulating renal membrane transport and hypertension. Curr Opin Nephrol Hypertens 2013; 22:163–169.

An outstanding review on the effects of EETs on sodium transport.

8. Wang WH, Zhang C, Lin DH, et al. Cytochrome P450 epoxygenase in the collecting duct is essential for the high K⁺ intake-induced antihypertensive effect. Am J Physiol Renal Physiol 2014; 307:F453–F460.

Evidence that CYP2C44 plays an essential role in maintaining normal blood pressure following an elevation in potassium intake.

9. Eid S, Maalouf R, Jaffa AA, et al. 20-HETE and EETs in diabetic nephropathy: a novel mechanistic pathway. PLoS One 2013; 8:e70029.

Recent study implicating a role of 20-HETE in diabetic nephropathy.

10. Chen G, Xu R, Wang Y, et al. Genetic disruption of soluble epoxide hydrolase is protective against streptozotocin-induced diabetic nephropathy. Am J Physiol Endocrinol Metab 2012; 303:E563–E575.
11. Wu CC, Gupta T, Garcia V, et al. 20-HETE and blood pressure regulation: clinical implications. Cardiol Rev 2014; 22:1–12.
12. Regner KR, Roman RJ. Role of medullary blood flow in the pathogenesis of renal ischemia–reperfusion injury. Curr Opin Nephrol Hypertens 2012; 21:33–38.
13. Klawitter J, Klawitter J, McFann K, et al. Bioactive lipid mediators in polygenic kidney disease. J Lipid Res 2013; 55:1139–1149.

This study provides evidence that urinary excretion of 20-HETE is correlated with the progression of PKD in humans.

14. Akbulut T, Regner KR, Roman RJ, et al. 20-HETE activates the Raf/MEK/ERK pathway in renal epithelial cells through an EGFR- and α-Src-dependent mechanism. Am J Physiol Renal Physiol 2009; 297:F662–F670.
Park F, Sweeney WE, Jia G, et al. Endothelial expression of human cytochrome

Liu H, Zhao Y, Nie D, et al. Androgen-sensitive hypertension associates

Basora N, Boulay G, Bilodeau L, et al. Production of 20-HETE and its

Gainer JV, Bellamine A, Dawson EP, et al. Increased renal proximal convoluted

Sinal CJ, Miyata M, Tohkin M, et al. Association of a functional cytochrome P450 4F2

45

Wang WH, Lu M, Hebert SC. Cytochrome P-450 metabolites mediate

Makita K, Takahashi K, Karara A, et al. Functional polymorphism in human

Benter IF, Yousif MH, Canatan H, et al. Endogenously produced 20-HETE modulates

Singh H, Cheng J, Deng H, et al. Association of common variants of CYP4A11

Urinary CYP eicosanoid excretion

Sudhahar V, Shaw S, Imig JD. Epoxyeicosatrienoic acids, 20-hydroxyeicosatetraenoic acid, and

Wei Y, Lin DH, Kemp R, et al. Targeted disruption of soluble epoxide

Ward NC, Tsai IJ, Barden A, et al. Correlation analysis and identification of

1062-4821

Hypertension and renal disease Fan et al.

Page 11 of 45

Hypertension and renal disease Fan et al.

Circulation 2006; 113:23–28.

Hypertension 2007; 49:687–694.

Am J Physiol 1996; 271:C103–C111.

Am J Physiol Renal Physiol 2011; 301:F672–F681.

Prehypertension 2009; 11:293–344.

Am J Physiol Renal Physiol 2009; 296:F586–F589.

Physiology 2007; 11:419–426.

Hypertension 2008; 51:1393–1398.

J Hypertens 2006; 24:765–771.

Circulation 2005; 111:63–69.

Circulation 2008; 21:1216–1223.

J Hypertens 2006; 22:19:1606–1612.

J Am Soc Nephrol 2009; 20:1263–1269.

J Hypertens 2005; 23:1929–1939.

Am J Physiol Regul Integr Comp Physiol 2012; 302:R1209–R1218.

J Am Soc Nephrol 2008; 19:1606–1612.

J Am Soc Nephrol 2010; 307:F533–F538.

J Am Physiol Renal Physiol 2009; 297:F740–F748.

J Am Physiol Renal Physiol 2009; 296:F586–F589.

J Gen Physiol 2004; 124:719–727.

J Am Physiol 2004; 294:F1680–F1686.

J Am Physiol 1996; 271:C103–C111.

Am J Physiol Renal Physiol 2013; 305:F1680–F1686.

J Am Physiol Renal Physiol 2013; 306:3368–344.

J Cardiovasc Pharmacol 2010; 56:3368–344.

Circulation 2005; 111:293–344.

The first study showing 20-HETE excretion is associated with the progression of renal injury in humans.

1. doi: 10.1016/j.prostaglandins.2014.08.002. [Epub ahead of print]

1. doi: 10.1093/ndt/gft729. [Epub ahead of print]

1. doi: 10.1002/hyp.10822.

1. doi: 10.1093/ndt/gft828. [Epub ahead of print]

1. doi: 10.1002/hyp.10822.

1. doi: 10.1002/hyp.10822.

1. doi: 10.1002/hyp.10822.
65. Capdevila JH, Pidkovka N, Mei S, et al. The Cyp2c44 epoxygenase regulates epithelial sodium channel activity and the blood pressure responses to increased dietary salt. J Biol Chem 2014; 289:4377–4386. Recent study showing that knockout of CYP2C44 increases ENaC activity and causes salt sensitive hypertension.

66. Nakagawa K, Holla VR, Wei Y, et al. Salt-sensitive hypertension is associated with dysfunctional Cyp4a10 gene and kidney epithelial sodium channel. J Clin Invest 2006; 116:1696–1702.

67. Imig JD, Hammock BD. Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases. Nat Rev Drug Discov 2009; 8:794–805.

68. Yu BN, Luo CH, Wang D, et al. CYP2C9 allele variants in Chinese hypertension patients and healthy controls. Clin Chim Acta 2004; 348:57–61.

69. Dahly-Vernon AJ, Sharma M, McCarthy ET, et al. Transforming growth factor-beta, 20-HETE interaction, and glomerular injury in Dahl salt-sensitive rats. Hypertension 2005; 45:643–648.

70. Williams JM, Sharma M, Arjaiah S, et al. Role of endogenous CYP450 metabolites of arachidonic acid in maintaining the glomerular protein permeability barrier. Am J Physiol Renal Physiol 2007; 293:F501–F505.

71. Eid S, Abou-Kheir W, Sabra R, et al. Involvement of renal cytochromes P450 and arachidonic acid metabolites in diabetic nephropathy. J Biol Regul Homeost Agents 2013; 27:693–703.

72. Eid AA, Gonn Y, Fagg BM, et al. Mechanisms of podocyte injury in diabetes: role of cytochrome P450 and NADPH oxidases. Diabetes 2009; 58:1201–1211.

73. Chen G, Wang P, Zhao G, et al. Cytochrome P450 epoxygenase CYP2J2 attenuates nephropathy in streptozotocin-induced diabetic mice. Prostaglandins Other Lipid Mediat 2011; 96:63–71.

74. Regnér KR, Zúk A, Van Why SK, et al. Protective effect of 20-HETE analogues in experimental renal ischemia reperfusion injury. Kidney Int 2009; 75:511–517.

75. Hofl U, Lukitsch I, Chaykovska L, et al. Inhibition of 20-HETE synthesis and action protects the kidney from ischemia/reperfusion injury. Kidney Int 2011; 79:57–65.

76. Romain RJ, Akbulut T, Park F, et al. 20-HETE in acute kidney injury. Kidney Int 2011; 79:10–13.

77. Liu Y, Webb HK, Fukushima H, et al. Attenuation of cisplatin-induced renal injury by inhibition of soluble epoxide hydrolase involves nuclear factor kappaB signaling. J Pharmacol Exp Ther 2012; 341:725–734.

78. Jarrar YB, Cha EY, Seo KA, et al. Determination of major UDP-glucuronosyltransferase enzymes and their genotypes responsible for 20-HETE glucuronidation. J Lipid Res 2014; 55:2334–2342. [Epub ahead of print] First paper to link polymorphisms in a 20-HETE glucuronosyltransferase to blood pressure in humans.

79. Cheng JW, Frishman WH, Aronow WS. Updates on cytochrome p450-mediated cardiovascular drug interactions. Dis Mon 2010; 56:163–179.

80. Zhou SF, Zhou ZW, Huang M. Polymorphisms of human cytochrome P450 2C9 and the functional relevance. Toxicology 2010; 278:165–188.

81. Totah RA, Retie AE. Cytochrome P450 2C8: substrates, inhibitors, pharmacogenetics, and clinical relevance. Clin Pharmacol Ther 2005; 77:341–352.

82. Knights KM, Rowland A, Miners JO. Renal drug metabolism in humans: the potential for drug–endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT). Br J Clin Pharmacol 2013; 76:587–602. Outstanding reference describing glucuronosyl transferase associated with CYP metabolites.