Inhibition of soluble epoxide hydrolase prevents renal interstitial fibrosis and inflammation

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Department of Cellular and Integrative Physiology, University of Nebraska Medical Center, Omaha, Nebraska; Department of Anatomy, Jeju National University School of Medicine, Jeju, Republic of Korea; Department of Biomedicine and Drug Development, Jeju National University, Jeju, Republic of Korea; Department of Pharmacology and Toxicology and Cardiovascular Center, Medical College of Wisconsin, Milwaukee, Wisconsin; Department of Entomology and Comprehensive Cancer Center, University of California, Davis, California; and Department of Internal Medicine, Section of Nephrology, University of Nebraska Medical Center, Omaha, Nebraska

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Kim J, Imig JD, Yang J, Hammock BD, Padanilam BJ. Inhibition of soluble epoxide hydrolase prevents renal interstitial fibrosis and inflammation. Am J Physiol Renal Physiol 307: F971–F980, 2014. First published August 27, 2014; doi:10.1152/ajprenal.00256.2014.—The pathological events that lead to renal interstitial fibrogenesis are incompletely understood. Epoxideicosatrienoic acid (EET), an arachidonic acid metabolite, has anti-inflammatory and profibroinolytic functions. Soluble epoxide hydrolase (sEH) converts EET to less active dihydroxyeicosatrienoic acid. Here, we tested the hypothesis that sEH deficiency would prevent tubulointerstitial fibrosis and inflammation induced by unilateral ureteral obstruction (UUO) in mouse kidneys. The loss of sEH enhanced levels of EET regioisomers and abolished tubulointerstitial fibrosis as demonstrated by reduced collagen deposition and myofibroblast formation at 3 and 10 days after UUO. The inflammatory response was prevented as demonstrated by decreased influx of neutrophil and macrophage, expression of inflammatory cytokines, and chemotactic factors in sEH-deficient UUO kidneys. Pharmacological inhibition of sEH also prevented inflammation and fibrosis after UUO. Next, we delved into the molecular mechanisms piloting the beneficial effects of sEH deficiency in renal fibrosis. UUO upregulated profibrotic factors associated with transforming growth factor (TGF)-β1/Smad3 signaling, oxidative stress, and NF-κB activation, and downregulated antifibrotic factors including peroxisome proliferator-activated receptor (PPAR) isoforms, especially PPAR-γ, but the loss of sEH prevented these adverse effects in UUO kidneys. Furthermore, administration of PPAR antagonists enhanced myofibroblast formation and activation of Smad3 and NF-κB p65, effects that were prevented by sEH deficiency in UUO kidneys. These data demonstrate that loss of sEH promotes anti-inflammatory and fibroprotective effects in UUO kidneys via activation of PPAR isoforms and downregulation of NF-κB, TGF-β1/Smad3, and inflammatory signaling pathways. Our data suggest the potential use of sEH inhibitors in treating fibrotic diseases.

chronic kidney disease; unilateral ureteral obstruction; PPAR isoforms; soluble epoxide hydrolase; tubulointerstitial fibrosis

CHRONIC KIDNEY DISEASE (CKD) is progressive, incurable, and ultimately fatal, either because of the consequences of kidney failure or an alarmingly high level of cardiovascular mortality in the CKD patient population (1). Regardless of the disease etiology, including hypertension and diabetes, tubulointerstitial fibrogenesis is the final common pathway in CKD that leads to disease progression and, ultimately, end-stage renal disease (ESRD). The past decade has seen great strides in our understanding of renal tubulointerstitial fibrosis, particularly as to how inflammation, fibroblast activation, tubular and microvascular injury contribute to fibrogenesis (3). However, pharmacological interventions based on the functions of many identified pathogenic factors have failed, are inadequate, or at best can slow progression to ESRD, and therefore development of effective drugs to treat renal tubulointerstitial fibrosis remains an elusive goal.

The eicosanoid metabolites of the arachidonic acid cascade play important roles in physiology including vasodilatory, anti-inflammatory, and antiapoptotic functions (8, 40). Cytochrome P-450 epoxygenases metabolize arachidonic acid into epoxyeicosatrienoic acid (EET), by catalyzing the epoxidation of the olefinic bonds of arachidonic acid, resulting in the production of four regioisomeric EETs: 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. Soluble epoxide hydrolase (sEH) enzyme converts the EETs to their corresponding less potent diols termed dihydroxyeicosatrienoic acids (DHETs) and therefore the sEH activity is thought to be a major determinant of EET bioavailability (48). Genetic deficiency of sEH increases EET levels in tissues and plasma, potentiates the effects of EETs, and thus elicits antihypertensive and anti-inflammatory effect (18). Loss of sEH is also shown to decrease glomerular injury and renal inflammation in rodent models of deoxycorticosterone (DOCA)-salt-induced hypertension (33, 34). Inhibiting the sEH enzyme enhances the beneficial cardiovascular properties of EETs (17). Loss of sEH also reduces renal injury in a CKD model of a type 1 diabetes (11); however, a role for sEH in the pathogenesis of renal interstitial fibrosis is not defined. We hypothesized that diminished availability of EETs could be a potential mechanism for the progression of tubulointerstitial fibrosis, and increasing the bioavailability of EETs is therefore a promising therapeutic strategy for CKD. In this study, we tested the premise that genetic deficiency of sEH may prevent fibrogenesis and inflammation in the unilateral ureteral obstruction (UUO) model of renal interstitial fibrogenesis.

MATERIALS AND METHODS

Renal fibrosis model. Male C57BL/6J mice aged 8 to 10 wk were purchased from Jackson Laboratories. Male sEH-knockout (KO) mice were previously reported (34). All mouse experiments were performed in accordance with the animal protocols approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center. UUO was conducted as previously reported...
Briefly, the mice were anesthetized with an intraperitoneal injection of a cocktail containing ketamine (200 mg/kg body wt) and xylazine (16 mg/kg body wt). After the left kidney was exposed through the left flank incision, the left ureter was ligated completely near the kidney pelvis using a 5–0 silk tie. Sham-operated mice underwent the same surgical procedure without the ureter ligation. Glomerulonephritis (GN) was developed as previously reported (25). Briefly, mice were immunized with 1 mg of normal sheep IgG (Jackson ImmunoResearch, West Grove, PA) in Freund’s complete adjuvant (Santa Cruz Biotechnology, Santa Cruz, CA) and injected intravenously with 100 µl of sheep anti-rat glomerular basement membrane (GBM) serum (ProtekteX, San Antonio, TX) 10 days later. Control mice underwent the same procedure without the injection of anti-GBM. For the pharmacological inhibition of sEH, trans-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (tAUCB; 2 or 10 mg/day) (41) or vehicle (10% DMSO in 0.5% methylcellulose) was administered by oral gavage beginning 3 days after UUO. For the pharmacological inhibition of PPARγ, a mixture of GW6471 (1 mg·kg body wt⁻¹·day⁻¹; R&D Systems, Minneapolis, MN) against PPARα, GSK0660 (1 mg·kg body wt⁻¹·day⁻¹; R&D Systems) against PPARβ/δ, and T0070907 (1.5 mg·kg body wt⁻¹·day⁻¹; R&D Systems) against PPARγ or vehicle (10% DMSO in 0.9% saline) was intra-peritoneally injected on the day of UUO surgery.

Collagen deposition. Collagen deposition was assessed by both Sirius red staining and hydroxyproline assay as previously described (26). The area of positive Sirius red staining was measured in five randomly chosen high-power (×200) fields per kidney using NIH Image J software.

Histology. Immunohistochemical staining of the kidneys was performed on paraffin sections as previously described (27). Briefly, 4% paraformaldehyde-fixed kidney sections were rehydrated and labeled with antibodies against α-smooth muscle actin (α-SMA; Sigma, St. Louis, MO), F4/80 (Abcam, Cambridge, MA), or polymorphonuclear neutrophil (PMN; Accurate, Westbury, NY). The sections were then incubated to peroxidase-conjugated secondary antibodies (Vector Laboratories, Burlingame, CA). The quantitative measurement of the immunohistochemical staining was carried out at a suitable magnification (×200; 62,500 µm²). The respective α-SMA- and F4/80-positive areas were measured in five randomly chosen fields per kidney using NIH Image J software. The number of PMN-positive cells in five randomly chosen fields per kidney was directly counted under light microscopy (Leica). Periodic acid-Schiff (PAS)-stained sections were used for tubular injury score as described previously (24). Histological damage of tubular injury was scored by percentage of tubules that displayed tubular necrosis, cast formation, and tubular dilation as follows: 0 = normal, 1 = <10%, 2 = 10 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, and 5 = >75%. Ten randomly chosen high-power (×200 magnification) fields per kidney were used for the counting.

Western blot. We performed electrophoresis of protein extracts using tris-glycine buffer systems and subsequent blotting as previously described (24). Membranes were incubated with antibodies against α-SMA (Sigma), fibronectin (Cedarlane, Hornby, Ontario, Canada), phosphorylated Smad3 (p-Smad3), phosphorylated NF-κB p65 (p-p65), poly(ADP-ribose) polymerase 1 (PARP1), caspase-3 (Cell Signaling, Beverly, MA), poly ADP-ribose (PAR; BD Pharmingen, San Jose, CA), intercellular adhesion molecule-1 (ICAM-1; Santa Cruz Biotechnology), TNF-α (Abcam), or sEH (Cayman). Peroxidase-conjugated secondary antibodies (Vector Laboratories) were applied, and a chemiluminescence reagent (PerkinElmer, Boston, MA) was used to detect proteins. Anti-β-actin antibody (Sigma) was used for loading controls on stripped membranes. The bands were quantified using Lab Works analysis software (Ultra-Violet Products, Cambridge, UK).

ELISA. The ratios of EETs to DHETs in the kidneys were assessed using EET/DHET ELISA immunoassay kit (DortoR&D, Detroit, MI). The levels of transforming growth factor (TGF)-β1, KC, macrophage inflammatory protein-2 (MIP-2), and monocyte chemotactic protein-1 (MCP-1) in the kidneys were measured using multiplex immunoassay (Millipore, Bedford, MA). The activities of peroxisome proliferator-activated receptor (PPAR) isofoms in the kidneys were measured using PPARα, β/δ, γ transcription factor assay kit (Cayman). The level of lipid hydroperoxide in the kidneys was measured using lipid hydroperoxide assay kit (Cayman).

Oxylipin profiling. Oxylipin concentrations in kidney tissues were measured by liquid chromatography/tandem MS analysis as previously described (47).

Statistical analyses. ANOVA was used to compare data among groups. Differences between two groups were assessed by two-tailed unpaired Student’s t-test. P values <0.05 were considered statistically significant.

RESULTS

Loss of sEH prevents EETs to DHETs conversion in UUO kidneys. To assess the role of sEH during renal interstitial fibrogenesis in vivo, we used a mouse model of obstructive nephropathy. UUO significantly increased the expression of sEH protein in wild-type (WT) mice at 3 and 10 days post-injury compared with that in sham-operated mice (Fig. 1A). No expression of sEH was observed in sEH-deficient mice at any of the time points studied. The ratio of EETs to DHETs was assessed by immunoassay. The ratio of both 11,12-EET/DHET...
and 14,15-EET/DHET decreased in WT compared with that in sEH-KO mice (Ephx2<sup>-/-</sup>) kidneys at 10 days after UUO. Levels of sEH-independent oxylipin metabolites showed no significant difference between WT and sEH-KO mice (Supplementary Table S1; the online version of this article contains supplemental data). These data indicate that the increased sEH activity reduced the ratio of EETs to DHETs in WT UUO kidneys consistent with its increased expression levels (Fig. 1B).

**Loss of sEH prevents renal fibrosis in UUO and GN models.** To determine whether sEH activation contributes to interstitial fibrosis induced by UUO, Collagen deposition was assessed by Sirius red staining and hydroxyproline measurement. During UUO, WT kidneys showed a time-dependent increase of collagen deposition as demonstrated by increased Sirius red-positive area (Fig. 2, A and B) and hydroxyproline level (Fig. 2C), whereas the loss of sEH markedly reduced the collagen deposition (Fig. 2, A–C). Tubulointerstitial expression of α-SMA, a marker of myofibroblast formation, was induced at 3 and 10 days in WT UUO-induced kidneys but was significantly diminished by the loss of sEH at both time points (Fig. 2, A, D, and E). Three and 10 days after UUO, fibronectin expression level in whole kidneys was also diminished by the loss of sEH (Fig. 2E). We also tested whether loss of sEH prevented renal interstitial fibrosis during GN, another model of renal fibrosis. During GN, collagen deposition in WT kidneys was time-dependently increased as demonstrated by

![Image](http://example.com/image.png)

**Fig. 2.** Loss of sEH prevents interstitial fibrogenesis induced by UUO. A: collagen deposition detected by Sirius red stain and α-smooth muscle actin (α-SMA) expression stained with α-SMA monoclonal antibody on kidney sections in WT or sEH-KO mice at 10 days after sham operation or UUO. Arrow heads indicate glomeruli. Scale bars = 50 μm. To demonstrate the specific staining of α-SMA in the myofibroblasts in the interstitial area, representative high magnification of the α-SMA staining regions from UUO-induced kidneys is shown in the bottom panels. B: percent of Sirius red-positive area on kidney sections. C: collagen content represented by hydroxyproline level in the kidneys. D: percent of α-SMA-positive area on the kidney sections. E: profibrotic expression of α-SMA and fibronectin in the kidneys evaluated by Western blot analyses. The bands were quantified using Lab Works analysis software. Error bars represent SD (n = 5).

*P < 0.05 vs. sham. #P < 0.05 vs. WT. §P < 0.05 vs. 3 days.
Sirius red-positive area (Fig. 3, A and B) and hydroxyproline level (Fig. 3C), whereas the loss of sEH significantly reduced the collagen deposition at 10 days of GN (Fig. 3). These data suggest that the loss of sEH has anti-fibrotic effect in different experimental models of CKD.

**Loss of sEH prevents TGF-β expression and its downstream signaling in UUO kidneys.** Since myofibroblast differentiation and activation by TGF-β signaling are critical events in renal fibrosis (6), we tested whether the TGF-β1 level is affected by sEH upregulation in WT UUO kidneys. The TGF-β1 level and its downstream signaling mediator p-Smad3 are significantly reduced in sEH-KO kidneys at 3 and 10 days after UUO, compared with those in WT kidneys (Fig. 4, A and B). These data suggest that sEH upregulation contributes to tubulointerstitial fibrogenesis via TGF-β1/p-Smad3 signaling.

**Loss of sEH diminishes infiltration of neutrophils and macrophages after UUO.** To determine whether sEH is implicated in renal inflammation in obstructive nephropathy, we next examined leukocyte influx and proinflammatory response in UUO kidneys. Three and 10 days after UUO, a prominent influx of both PMN-positive neutrophil and F4/80-positive macrophage occurred in WT kidneys, but the loss of sEH significantly prevents inflammatory cell infiltration in UUO kidneys (Fig. 5).

**Loss of sEH diminishes expression of inflammatory cytokines and chemotactic factors after UUO.** To determine whether sEH alters the inflammatory milieu in the UUO-induced kidney, we assessed the levels of various inflammatory molecules. Loss of sEH decreased levels of keratinocyte-derived chemokine (KC), MIP-2, and MCP-1 proteins, potent leukocyte chemotactic factors upregulated by UUO (Fig. 6A). Since these chemotactic factors can be transactivated by NF-κB transcription factor (38, 43, 45), we assessed NF-κB activation by quantifying p-p65 in UUO kidneys. The level of p-p65 was significantly reduced in sEH-KO kidneys during UUO compared with that in WT kidneys, respectively (Fig. 6B). Expression levels of NF-κB target gene proteins, TNF-α and ICAM-1, were increased in WT kidneys, but were prevented by the loss of sEH during UUO (Fig. 6B). These data suggest that sEH upregulation exacerbates renal inflammation at least partly through an NF-κB-dependent signaling pathway during interstitial fibrogenesis.

**Pharmacological inhibition of sEH post-UUO attenuates increase of fibrotic and inflammatory protein expression.** To determine whether treatment with tAUCB, a pharmacological inhibitor of sEH, during UUO attenuates renal fibrosis, we measured fibrotic protein expression. Treatment with tAUCB beginning 3 days post-UUO significantly reduced α-SMA and fibronectin expression compared with vehicle-treated animals (Fig. 7A). We also assessed the expression of inflammatory proteins in kidneys with or without treatment with tAUCB during UUO. UUO-induced expression of TNF-α and ICAM-1 was significantly diminished by pharmacological inhibition of sEH at 10 days after the injury (Fig. 7B). These data suggest that sEH inhibitors may be used as a prophylactic agent to attenuate progression of fibrosis in CKD.

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**Fig. 3.** Loss of sEH prevents interstitial fibrosis induced by glomerulonephritis (GN). A: collagen deposition detected by Sirius red stain and α-SMA expression stained with α-SMA monoclonal antibody on kidney sections in WT or sEH-KO mice at 10 days after nonadministration or administration of anti-glomerular basement membrane (GBM) serum. Scale bars = 50 μm. B: percent of Sirius red-positive area on kidney sections. C: collagen content represented by hydroxyproline level in the kidneys. Error bars represent SD (n = 4). *P < 0.05 vs. sham. ** P < 0.05 vs. WT. § P < 0.05 vs. 3 days.

**Fig. 4.** Loss of sEH reduces transforming growth factor (TGF)-β signaling during UUO. A: kidney TGF-β1 level in WT or sEH-KO mice after sham operation or UUO was measured by multiplex immunoassay. B: Smad3 phosphorylation (p-Smad3) in the kidneys was analyzed by Western blot. The bands were quantified using Lab Works analysis software. Error bars represent SD (n = 5). *P < 0.05 vs. sham. ** P < 0.05 vs. WT. § P < 0.05 vs. 3 days.
Loss of sEH diminishes cell death and oxidative stress after UUO. Since inflammation and fibrogenesis can be initiated by cell death (37) and vice versa, we assessed tubular injury and cell death in UUO-subjected kidneys with or without the sEH deficiency. Tubular injury score based on PAS-stained kidney sections was increased at 3 and 10 days after UUO, but the loss of sEH significantly prevented the tubular injury in UUO kidneys (Fig. 8, A and B). The loss of sEH also reduced UUO-induced tubular cell death including both necrosis as demonstrated by PARP1-dependent increased PAR formation and PARP1 expression, and apoptosis demonstrated by cleaved PARP1 and caspase-3 expression (Fig. 8C). To explore whether the loss of sEH contributes to decreased oxidative stress during UUO, we next evaluated lipid peroxidation in UUO kidneys in WT and sEH-KO mice. UUO increased lipid peroxidation as represented by the increased level of lipid hydroperoxide in WT kidneys at 3 and 10 days, whereas the loss of sEH ameliorated lipid peroxidation induced by UUO (Fig. 8D), suggesting that sEH upregulation induces oxidative stress in WT UUO-induced kidneys, potentially leading to the progression of tubular injury and cell death.

sEH is required for PPAR inactivation during renal fibrosis. PPAR isoforms play a protective role in renal interstitial fibrosis (13, 23). The activity of PPAR isoforms was time-
bands were quantified using Lab Works analysis software. Error bars represent SD.

**DISCUSSION**

Fibrosis is an essential process for survival; however, disturbed fibrosis leads to a variety of deleterious events. Thus, it is no surprise that the pathophysiological events that lead to renal interstitial fibrogenesis are complex and involve several independent and overlapping cellular and molecular signaling pathways. Endothelial cell activation, leukocyte infiltration, tubular cell injury, capillary rarefaction, and pericyte and myofibroblast activation all play important roles in the pathogenesis of renal fibrosis after an inciting insult to the renal parenchyma (2, 7, 30, 44). Traditionally, TGF-β signaling has been regarded as a central mediator of tubulointerstitial fibrosis (4, 5). Recently, TGF-β-independent signaling via other molecules including NF-κB and angiotensin II is also implicated in renal fibrosis (19, 39). However, the molecular signaling pathways that activate these overlapping pathways to elicit renal fibrogenesis are not defined.

In the present study, we tested the effect of the seH deficiency on the progression of renal failure in mouse UUO model. Our data indicate that the loss of seH is beneficial in attenuating the inflammation and interstitial fibrogenesis after UUO. UUO decreased the ratios of EETs to DHETs in WT mouse kidneys that were restored to normal levels by the loss of seH. The loss of seH significantly reduced the intrarenal levels of inflammatory cytokines and chemokines, and the infiltration of both neutrophils and macrophages into the renal parenchyma. The loss of seH by a genetic approach prevented collagen, fibronectin, and α-SMA deposition in the UUO-induced kidneys. The loss of seH also prevented histological damage, oxidative stress, PARP1 and NF-κB activation, and significantly increased the activities of PPARs, especially the level of PPARγ. Taken together, increasing the levels of EETs by the seH deficiency prevents renal interstitial inflammation and fibrogenesis in the mouse UUO model. Our data suggest that the renal seH activation decreases the EET levels to elicit pathologically relevant inflammatory responses and interstitial fibrogenesis, which are mediated by downregulation of PPAR activity.

PPARs are ligand-activated transcription factors. EETs bind to the ligand-binding domain of PPARγ with high affinity (32). Several independent reports have demonstrated that PPARγ is a negative regulator of profibrotic signaling and blocks matrix deposition and fibrogenesis in diabetic glomerulosclerosis (36) and CCl4-induced liver fibrosis (29). Our data indicate that PPAR levels are downregulated in UUO kidneys. The seH deletion induces the levels of PPARs and prevents UUO-induced fibrosis. However, the molecular signaling pathways by which PPARs attenuate UUO-induced fibrosis are not well-characterized. A role for PPARγ in regulating reactive oxygen species (ROS) levels in the hypothalamus in high fat-fed mice was previously reported (9). The anti-inflammatory effect of PPARγ on LPS-induced pulpal inflammation is also due to decreasing ROS levels (28). Our data indicating
that the sEH deficiency downregulates oxidative stress in UUO kidneys are consistent with these reports.

ROS generated during UUO contributes to oxidative damage to DNA (27). Under conditions of increased DNA damage, PARP1 is hyperactivated and can act as a promoter-specific coactivator of NF-κB in vivo independent of its enzymatic activity (14, 15). PARP1 binds directly to both subunits of NF-κB (p65 and p50) and promotes NF-κB-dependent transcription. Our data indicate that the sEH deficiency inhibits PARP1 activation in UUO-induced mice kidneys. Furthermore, the sEH deficiency prevents NF-κB activation and the inflammatory response. These data concur with earlier reports showing that sEH inhibition attenuated myocardial NF-κB activation in mice 6 wk after cardiac hypertrophy induced by UUO 10 d in Ephx2−/−UUO 10 d in WT

Fig. 8. Loss of sEH suppresses tubular cell damage during interstitial fibrogenesis. A: periodic acid-Schiff (PAS) stain on kidney sections in WT or sEH-KO mice at 10 days after sham operation or UUO. Scale bars = 50 μm. B: tubular injury score represented by PAS stain in the kidneys. C: protein expression of poly ADP-ribose (PAR), poly(ADP-ribose) polymerase 1 (PARP1), cleaved PARP1, and cleaved caspase-3 in the kidneys using Western blot. The bands were quantified using Lab Works analysis software. D: lipid peroxidation indicated by lipid hydroperoxide level in the kidneys using lipid hydroperoxide assay kit. Error bars represent SD (n = 5). *P < 0.05 vs. sham. #P < 0.05 vs. WT. §P < 0.05 vs. 3 days.

Fig. 9. Loss of sEH restricts the reduction of peroxisome proliferator-activated receptors (PPARs) activity induced by UUO. The activity of PPAR isoforms in kidneys in WT or sEH-KO mice after sham operation or UUO was assayed using PPARα, β/δ, γ transcription factor assay kit. Error bars represent SD (n = 6). *P < 0.05 vs. sham. #P < 0.05 vs. WT. §P < 0.05 vs. 3 days.
thoracic aortic constriction (46). Similarly, NF-κB activation in the kidney was significantly attenuated in sEH-KO mice with DOCA-salt-induced hypertension (34). As we previously reported (25), in the absence of PARP1, NF-κB mediated inflammatory mediators TNF-α and ICAM-1, and attenuated infiltration of inflammatory cells and the production of cytokines leading to reduced tubulointerstitial lesion and fibrosis in UUO-induced mouse kidneys.

In our data, sEH deficiency downregulated TGF-β1 level in the UUO kidneys. However, the EET-induced signaling pathways that lead to TGF-β1 downregulation remain undefined. A number of reports implicated a direct role for PPARγ in modulation of TGF-β/Smad3 signaling. The PPARγ agonist troglitazone attenuates UUO-induced renal interstitial fibrosis and inflammation through suppression of TGF-β1 expression (22). Ligand-activated PPARγ prevents TGF-β-induced collagen synthesis via sequestration of essential coactivator p300 from TGF-β-induced p-Smad complex on the collagen gene promoter (12). It remains to be determined whether EETs may attenuate TGF-β/Smad3 stimulation via PPARγ activation after UUO.

Our data are in contrast to a previous reported study in a hypertensive 5/6-Nx model of CKD where sEH inhibition did not delay disease progression but rather resulted in significant increase in glomerulosclerosis, tubulointerstitial damage index, and an increase in albuminuria (21). In previous reports, the antihypertensive effect of sEH inhibition was implicated for decreased renal inflammation and injury in rat and mouse models, such as spontaneously hypertensive rats, angiotensin II-induced hypertension, and DOCA-induced and salt-sensitive hypertension (16, 20, 33). It should be emphasized that inflammation and fibrogenesis observed in our sEH-deficient UUO mouse model are independent of changes in blood pressure. The differences in the results could be due to the model and the strain of mice as the antihypertensive effect in sEH-KO mice was lost after backcrossing on the C57BL/6 background (10, 34). The effect of sEH inhibition on renal protection independently of its effect on lowering blood pressure was previously reported in streptozotocin-induced diabetic mice (11) and in DOCA-salt hypertension (34). Our data are also consistent with a recent report demonstrating the beneficial effects of sEH inhibitors on adverse cardiac remodeling in ischemic cardiomyopathy and pressure-overload hypertrophy (42). On the other hand, in a recent report, pharmacological inhibition of sEH attenuated collagen deposition in angiotensin II-treated heart ventricles, but genetic inhibition of sEH conversely aggravated the effect (31) showing differing effects of pharmacological and genetic inhibition of sEH on myocardial fibrosis. In our data, both pharmacological and genetic inhibition had the same preventive effect on collagen deposition in the UUO model of renal fibrosis. The discrepancy may be due to the different sEH pathways activated during fibrogenesis and inflammation in hearts and kidneys.

Collectively, these studies as summarized in Fig. 11 demonstrate that increased EETs after the sEH deficiency significantly prevent histological damage, oxidative stress, and increased levels of PPAR, especially that of PPARγ. Furthermore, the increased levels of EETs prevented pathologically relevant inflammatory responses including reduced levels of inflammatory cytokines and chemokines, leukocyte infiltration, and...
adverse renal remodeling in the UUO kidneys. Our data demonstrate that EET promotes the anti-inflammatory and fibroprotective effects in UUO kidneys via activation of PPAR isoforms and downregulation of NF-κB, TGF-β1/Smad3, and inflammatory signaling pathways.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.K. and B.J.P. conception and design of research; J.K. and J.Y. performed experiments; J.K. analyzed data; J.K., J.D.I., B.D.H., and B.J.P. edited and revised manuscript; J.K. and J.Y. performed experiments; J.K. analyzed data; J.K., J.D.I., B.D.H., and B.J.P. approved final version of manuscript.

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