Soluble epoxide hydrolase is involved in the development of atherosclerosis and arterial neointima formation by regulating smooth muscle cell migration

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Submitted 22 April 2015; accepted in final form 18 August 2015

Wang Q, Huo L, He J, Ding W, Su H, Tian D, Welch C, Hammock BD, Ai D, Zhu Y. Soluble epoxide hydrolase is involved in the development of atherosclerosis and arterial neointima formation by regulating smooth muscle cell migration. Am J Physiol Heart Circ Physiol 309: H1894–H1903, 2015. First published October 9, 2015; doi:10.1152/ajpheart.00289.2015.—Epoxyeicosatrienoic acids (EETs), derived from arachidonic acid, have several beneficial effects on cardiovascular disease. Soluble epoxide hydrolase (sEH) metabolizes EETs to less active diols, thus diminishing their biological activity. sEH inhibitors can suppress the progression of atherosclerotic lesions in animal models. However, the regulation of sEH in vascular smooth muscle cells (VSMCs) and role of sEH in patients with atherosclerosis have not been evaluated. We hypothesize that sEH in VSMCs plays a pivotal role in atherosclerosis and injury-induced neointima formation. In this study, sEH expression in human autopsy atherosclerotic plaque was determined by immunohistochemistry. In cultured rat and human VSMCs, the phenotypic switching marker and sEH expression induced by platelet-derived growth factor-BB (PDGF-BB) were examined by Western blot analysis. Carotid-artery balloon injury was performed after adenovirus-mediated overexpression of sEH or oral administration of a potent sEH inhibitor in Sprague-Dawley rats. sEH was highly expressed in VSMCs of the intima and media within human atherosclerotic plaque. In vitro, PDGF-BB upregulated the expression in VSMCs after transcription and promoted cell proliferation and migration; the latter effect could be largely attenuated by an sEH inhibitor. Adenovirus-mediated overexpression of sEH could mimic the effect of PDGF-BB and induce VSMC proliferation and migration. In vivo, the sEH inhibitor led to a significant decrease in injury-induced neointima formation in a rat carotid-artery injury model. These data establish the effect of sEH expression on atherosclerotic progression and vascular remodeling after injury, thus identifying a novel integrative role for sEH in VSMC phenotypic modulation and migration. Blocking sEH activity may be a potential therapeutic approach for ameliorating vascular occlusive disease.

NEW & NOTEWORTHY

In this study, we found that soluble epoxide hydrolase (sEH) was highly expressed in vascular smooth muscle cells (VSMCs) of human atherosclerotic plaque and in rat neointima. Inhibition of sEH largely attenuated platelet-derived growth factor-BB-induced VSMC migration in vitro and injury-induced neointima formation in vivo, which suggests that sEH inhibition may be a potential therapeutic target for cardiovascular diseases.

EPOXYEICOSATRIENOIC ACIDS (EETs), derived from arachidonic acid, have several beneficial effects on cardiovascular homeostasis, including the ability to hyperpolarize vascular smooth muscle cells (VSMCs), dilate coronary arteries, and suppress adhesion molecules. Soluble epoxide hydrolase (sEH) metabolizes EETs to their corresponding dihydroxyeicosatrienoic acids, which limits the availability of EETs (16). Several models of atherosclerosis have suggested that inhibition of sEH, with an ensuing decrease in EET degradation, may attenuate atherosclerosis development and aneurysm formation (3, 14, 15, 19, 25). Treatment with sEH inhibitors also attenuated cardiovascular hypertrophy in rats with angiotensin II treatment and in a murine model with transverse aortic constriction (21). However, the involvement of sEH in the development of human cardiovascular disease is unclear.

VSMCs, a major blood vessel cell type, play major roles in maintaining vessel integrity and function. The migration of VSMCs from media to intima is pivotal for intimal thickening in cardiovascular disease. VSMCs in adult animals are plastic and retain their ability to switch rapidly from a contractile excitable phenotype to a migratory synthetic phenotype in response to injury (11). This modulation is the common fundamental biological mechanism of VSMC-related cardiovascular diseases such as atherosclerosis and neointimal formation after arterial injury. A characteristic of synthetic VSMCs is that they have low levels of differentiation marker proteins, including α-smooth muscle actin (α-SMA) and smooth muscle protein 22α (SM22α), which are required for the VSMC normal contractile function (12, 13).

Given the important role of VSMCs in atherosclerosis and neointima formation after arterial injury and the effect of sEH/EETs on the cardiovascular system, we hypothesize that sEH in VSMCs plays a pivotal role in vascular remodeling. In the current study, we investigated the role of VSMC sEH in atherosclerotic and injury-induced neointima formation. We found that sEH was highly expressed in VSMCs of human atherosclerotic plaques. In vitro, sEH played a critical role in VSMC migration by inhibiting platelet-derived growth factor-BB (PDGF-BB)-induced differentiation. Pharmacological inhibition of sEH in a balloon-injured carotid artery model decreased neointima formation and injury-induced VSMC-specific gene expression. sEH may be a potential therapeutic...
target for attenuating VSMC dedifferentiation and migration under pathological conditions.

**MATERIALS AND METHODS**

**Reagents.** Cycloheximide (CHX) and Hoechst stain reagent were from Sigma-Aldrich (St. Louis, MO). PDGF was from R&D Systems (Minneapolis, MN). Primary antibodies for sEH (sc-25797), osteopontin (OPN, sc-21742), GAPDH, CD68 (sc-9139), polyperoxidase-anti-mouse/rabbit IgG were from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies against smooth muscle α-actin (AA132) were from Beyotime Institute of Biotechnology (Beijing, China), and DyLight 488-labeled goat anti-rabbit IgG (H+L) and DyLight 594-labeled goat anti-mouse IgG (H+L) were from Zhongshan Golden Bridge (Beijing, China).

**Human samples and analysis.** Autopsy case records from 2001 to 2010 at the Verification Centre of Forensic Medicine affiliated with Shantou University were reviewed, from which 97 formalin-fixed paraffin embedded coronary or coronary artery sections were chosen and stained with hematoxylin and eosin (H&E) to evaluate the degree of atherosclerosis. On the basis of H&E staining, sections were separated into two groups: one nondiseased and one with atherosclerotic plaque. Ponceau/Victoria blue (P/VB) was used to stain the elastic fibers surrounding vessels and proteins in cells, respectively. Sixty-one samples exhibited atherosclerotic plaques and 36 showed nonplaque arteries. The age and sex of individuals in each group are listed in Table 1. Analysis of sEH location and intensity was per-

**Table 1. Age and gender information of autopsy samples**

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<th>Group</th>
<th>Age</th>
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<td>Nondiseased vessel, n = 36</td>
<td>18–60</td>
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<td>16</td>
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<td>Atherosclerotic plaques, n = 61</td>
<td>24–77</td>
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Fig. 1. Soluble epoxide hydrolase (EH) protein level is upregulated in atherosclerotic lesions of human coronary arteries. A: hematoxylin and eosin (H&E) staining of human coronary artery sections (nondiseased vessel, n = 36; atherosclerotic plaque, n = 61). B: representative immunohistochemical analysis of atherosclerotic patient coronary artery cross sections; a–d: immunostaining with Ponceau/Victoria blue (P/VB) and antibodies for soluble EH (sEH), α-smooth muscle actin (α-SMA), and CD68 (bar = 500 μm); e–h: higher amplification of boxes (bar = 100 μm). C: immunohistochemical staining with P/VB, sEH (arrows) and α-SMA. Top, bar = 100 μm; bottom, higher magnification of boxes, bar = 50 μm. D: quantification of sEH in intima and media. Nondiseased vessels, n = 36; atherosclerotic plaque, n = 61; data are means ± SE, **p < 0.001, media of nondiseased vessel group vs. media of atherosclerotic plaque group. Bar = 100 μm.
formed in a blinded fashion. The average density (integral optical density/area) was measured with Image-Pro Plus 6.0, and the result was determined as the sum of five different fields of each section. The level of sEH average density was normalized to that of nondiseased vessels in intima. The study was carried out in accordance with the Declaration of Helsinki and The Belmont Report (Washington, DC: U.S. Department of Health & Human Services, 1979), and was approved by the Ethics Committee of Shantou University Medical College.

Animal artery injury. Male Sprague-Dawley rats (210 to 230 g) were housed in a temperature-controlled room with a 12-h:12-h light-dark cycle and had free access to standard chow and water. The sEH inhibitor 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoromethoxy-phenyl)-urea (TUPS) was designed and synthesized in Bruce Hammock’s laboratory at Columbia University in New York. When compared with early generations of competitive sEH inhibitors, TUPS exhibits high potency (IC50 = 7.0 nM in rat), excellent oral availability, and pharmacokinetic properties (1). TUPS was dissolved and delivered at 0.65 mg·kg−1·day−1 in drinking water for 10 or 14 days as was previously reported (3), starting 7 days before surgery. A vehicle was administered to the control group. At 3 or 7 days after carotid artery injury, all animals (n = 7 to 9 per group) were anesthetized by CO2, and arteries were harvested. The protocols in the investigation followed those in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, Publication No. 85-23, Revised). All animal experimental protocols were approved by the Shantou University Institutional Animal Care and Use Committee.

Surgery was performed as described (20). Briefly, rats were anesthetized by injection of pentobarbital sodium (30 mg/kg ip). A balloon catheter of 1.5 mm in diameter (Medtronic, Minneapolis, MN) was introduced through the left external carotid artery and advanced 4 cm toward the thoracic aorta. The balloon was distended and pulled back to the bifurcation with constant rotation. This procedure was repeated two more times to ensure complete endothelial denudation. Contralateral arteries were harvested and subjected to histological analysis.

Fig. 2. Platelet-derived growth factor (PDGF) induces sEH expression in rat and human vascular smooth muscle cells (VSMCs). Rat VSMCs were treated with PDGF-BB for 24 h at the indicated dose (A) or 20 ng/ml PDGF for different times (B). Western blot analysis of sEH protein expression and quantification. *P < 0.05, **P < 0.01 compared with control. A paired t-test was performed. Human VSMCs were treated with PDGF-BB for 24 h at the indicated dose (C) or 20 ng/ml of PDGF for different times (D). Quantification of sEH protein expression is shown. *P < 0.05, **P < 0.01 compared with control. A paired t-test was performed. E: rat VSMCs were incubated with PDGF-BB at the indicated dose for 24 h. RT-PCR analysis of mRNA levels of sEH and VSMC differentiation markers was performed. Messenger RNA levels were normalized to those of GAPDH. *P < 0.05, **P < 0.01 compared with control. A t-test was performed. F: rat VSMCs were transfected with human sEH promoter-Luc plasmids (−1,000 and −471 bp) and treated with 20 ng/ml of PDGF-BB for 24 h. β-Gal plasmid was used as an internal control. Data are means ± SD of 3 independent experiments.

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00289.2015 • www.ajpheart.org
eral carotid arteries underwent a similar operation without injury and served as sham-operated controls.

**Morphometric analysis of rat carotid arteries.** Rat arteries were perfusion-fixed with 4% paraformaldehyde. Briefly, cryosections (6 μm thick, 350 μm apart) were taken from the middle portion of the balloon-injured segment, and eight sections of each sample were analyzed by H&E staining and with Spot Image software (Diagnostic Instruments, Australia). The intima and media areas and the circumference of the external elastic lamina were determined, and the ratio of intima to media area was calculated.

**VSMC culture.** Rat VSMCs were isolated from thoracic aortas of male Sprague-Dawley rats (180 to 200 g) as described (9) and maintained in DMEM (Gibco) containing 20% fetal bovine serum (FBS) (Highchone). Rats were euthanized with an overdose of pentobarbital sodium (100 mg/kg ip) before the aorta was excised. Purity of VSMCs was verified by anti-α-actin antibody staining, and cells between passages 2 to 4 were made quiescent by incubating in serum-free DMEM for 48 h at 37°C in a humidified atmosphere containing 5% CO₂ for in vitro experiments unless stated otherwise. A human VSMC cell line (HA-VSMC) was purchased from FuDan IBS Cell Center and cultured in DMEM/F12 with 10% FBS (Gibco), 0.05 mg/ml vitamin C (Sigma), 0.03 μg/ml EGF (Pr imeGene Bio Tech, Shanghai, China), 0.01 mg/ml insulin (Sigma), 0.01 mg/ml transferrin (Sigma), and 10 ng/ml sodium selenite (Sigma).

**Adenovirus infection.** The protocol of VSMCs infected with adenovirus sEH (Ad-sEH) or adenovirus-mediated green fluorescent protein (Ad-GFP) has been described elsewhere (3, 18, 23). sEH and β-galactosidase (Ad-GFP) have been described elsewhere (3, 18, 23). The adenovirus was used for transient transfection. VSMCs at 80% confluence were incubated with the primary antibodies for sEH, GAPDH, and β-galactosidase (1:200; Promega, Madison, WI). Protein concentration was determined by the bicinchoninic acid (BCA) Protein Assay Kit (

**Western blot analysis.** VSMCs were washed with PBS and lysed in RIPA buffer supplemented with protease inhibitor cocktail (NovasyGen, China). Protein concentration was determined by the bicinchoninic acid protein assay kit (NovasyGen). Equal amounts of protein were resolved by 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Millipore, Germany), which were incubated with the primary antibodies for sEH, α-SMA, OPN, and GAPDH, then corresponding secondary antibodies; blots were developed by use of Super ECL Plus Detection Reagent (Applygen, China).

**Transient transfection and luciferase activity assay.** Plasmids of the human sEH promoter, sEH-1000bp-Luc and sEH-471bp-Luc, were used for transient transfection. VSMCs at 80% confluence were transfected with 1 μg of sEH promoter and 0.5 μg of CMV-β-galactosidase by use of a Jet PEI (Polyplus, San Marcos, CA) for 24 h. Cells were treated with 20 ng/ml of PDGF-BB for another 24 h, and then lysed and collected for luciferase activity assay (Luciferase Reporter Assay System, Promega).

**Protein half-life measurements.** VSMCs were pretreated with CHX (100 μg/ml) for 30 min and then incubated with or without PDGF (20 ng/ml). Cells were harvested at 0, 1, 2, 4, and 8 h, and the amount of protein was detected by Western blot analysis. The kinetic constant and half-life of sEH protein with and without PDGF-BB were determined by linear regression analysis.

**In vitro scratch assay and transwell migration assay.** For scratch-induced wound healing assay, rat or human VSMCs were cultured until they were 90% confluent. The monolayers were scratched with a p200 pipette tip as described (10) and pretreated with TUPS (1 μM) or 14,15-EET (100 nM) for 30 min and then incubated with 20 ng/ml of PDGF-BB or PBS for 24 h in serum-free medium. The farthest distance that cells migrated from the wound edge was measured (average of 5 independent microscopic fields for each of 3 independent experiments). Analysis of VSMC invasion involved a modified Boyden chamber coated with an 8-μm barrier of reconstituted basement-membrane protein similar in composition to that surrounding VSMCs in vivo (Millipore, Billerica, MA).

**Assays of sEH activity.** EET and 14,15-dihydroxyeicosatetraenoic acid (DHET) were measured as previously reported (26). Briefly, cell lysates were spiked with 1 μl of internal standard mixture (5 μg for each internal standard). The Waters Oasis-HLB cartridges (1 ml volume) were used for solid-phase extraction. The elutions were further dried and redissolved in 30% acetonitrile. 14,15-EET and its metabolite of sEH, 14,15-DHET, were measured with ultra-high-performance liquid chromatography (UPLC; Waters, Milford, MA) and a 5500 QTRAP mass spectrometer (AB Sciex, Foster City, CA) equipped with a Turbo Ion Sprayelectrospray ionization source (liquid chromatography coupled with tandem mass spectrometry; LC-MS/MS) as described previously (26). The DHET-to-EET ratio was used to indicate sEH activity.

**Statistical analysis.** Results were analyzed by two-tailed Student’s t-test or one-way or a two-way ANOVA and linear regression as indicated, by use of GraphPad Prism software. A Bonferroni test was used to test for significant post hoc differences revealed by ANOVA. A Pearson χ² test was used to compare sEH positivity in the intima and media of nondiseased and atherosclerotic tissue by use of SPSS v11.5 (SPSS Inc, Chicago, IL). P < 0.05 was considered statistically significant.

![Fig. 3. PDGF increases the stabilization of sEH protein in rat VSMCs. A: VSMCs were serum-starved for 48 h, then treated with cycloheximide (CHX) (100 μg/ml) for the indicated time, with or without PDGF-BB (20 ng/ml). Western blot analysis of sEH protein level was normalized to GAPDH. Data are representative of 5 experiments. B: the slope of the decay line was calculated by standard linear regression analysis (P < 0.0001).](http://ajpheart.physiology.org/ by 10.2203.32.274 on October 15, 2017)
Fig. 4. Migration of human VSMCs induced by PDGF was partly reversed by the sEH inhibitor 1-(1-methanesulfonyl-piperidin-4-yl)-3-(4-trifluoro-methoxy-phenyl)-urea (TUPS) or epoxideicosatrienoic acid (14,15-EET). A and B: confluent human VSMC monolayers were preincubated with TUPS (1 μM) or 14,15-EET (100 nM) for 30 min and then treated with 20 ng/ml of PDGF. Scratch assay was performed. Images were taken at ×100 magnification. Data are means ± SD of 5 experiments. *P < 0.05, **P < 0.01 compared with control; #P < 0.05 compared with PDGF alone. C: human VSMCs were seeded in the upper chamber of transwells. PDGF-BB (20 ng/ml) was added in the lower chamber as a chemoattractant with TUPS (1 μM) or 14,15-EET (100 nM). Magnification is ×400; D: the mean cell number migrating across the filter was quantified in five randomly chosen fields. E: human VSMCs were incubated with or without TUPS (1 μM) and treated with 20 ng/ml PDGF for 24 h, after which 14,15-EET and 14,15-dihydroxyicosatrienoic acid (14,15-DHET) were determined with LC/MS-MS and the DHET-to-EET ratio was calculated. Data are means ± SD and analyzed by ANOVA from 5 independent experiments. *P < 0.05, **P < 0.01 compared with control; #P < 0.05, ##P < 0.05 compared with PDGF alone.
RESULTS

sEH protein is highly expressed in VSMCs in human atherosclerotic lesions. To investigate the role of sEH in atherosclerosis, we studied its expression in human coronary artery sections with polyclonal rabbit anti-human sEH antibody. Sections from 97 autopsy cases were divided into two groups: sections containing nondiseased vessels and sections containing atherosclerotic plaques (Fig. 1A) according to H&E staining of samples and pathology diagnosis.

To further identify the sEH-positive cells, we immunostained human sections with P/VB and antibodies for sEH, α-SMA, and CD68. The expression of sEH corresponded to that of α-SMA rather than CD68 (Fig. 1B), which suggests that sEH was predominantly expressed in VSMCs in the intima and media (Fig. 1C). Moreover, the density of sEH was comparable in intima, which was significantly increased in the media of atherosclerotic lesions (Fig. 1D). Thus high sEH expression in intima, which was significantly increased in the media of atherosclerotic lesions.

PDGF-BB posttranscriptionally upregulated sEH expression in rat primary and human VSMCs. To explore a potential role for sEH in VSMC migration, we measured the expression of sEH in response to the VSMC migration inducer PDGF-BB. Rat and human primary VSMCs were treated with different doses of PDGF-BB for 24 h. sEH protein expression was induced by PDGF-BB at 10 ng/ml and peaked at 40 ng/ml (Fig. 2, A and C). In addition, PDGF treatment increased sEH protein levels at 8 and 4 h, in rat and human cells, respectively, which lasted for at least 24 h (Fig. 2, B and D). Thus PDGF induced sEH expression in both a dose- and time-dependent manner. The increase in sEH expression paralleled that of a reduced expression of contractile SMC markers such as α-SMA, calponin 1, and SM22α. However, the mRNA levels of sEH and luciferase activity of the sEH promoter were not changed by PDGF-BB in VSMCs (Fig. 2, E and F). Therefore, PDGF-BB-induced regulation of sEH occurred posttranscriptionally.

Because sEH was regulated by PDGF at the protein level, we next tested whether PDGF-BB enhanced the stability of sEH protein. We used CHX to block protein synthesis and measured the degradation of sEH in VSMCs. Soluble degradation was slower with PDGF-BB than control treatment (Fig. 3A). CHX treatment produced an sEH half-life of 2.5 ± 0.5 min in control cells and 4.6 ± 0.2 min in PDGF-treated VSMCs (Fig. 3B).

![Fig 5. sEH overexpression promotes VSMC migration.](http://ajpheart.physiology.org/)
sEH promoted the migration of VSMCs. We next investigated the function of sEH in the migration of VSMCs. In vitro scratch wound assay revealed that PDGF-BB-stimulated human VSMC migration could be reversed by addition of the sEH inhibitor TUPS and 14,15-EET (Fig. 4A). When compared with PDGF-BB treatment alone, TUPS treatment reduced the mean migration distance at 6 and 12 h after scratch wounding to 72 and 76% in human VSMCs, respectively (Fig. 4B). In addition, with transwell assay and PDGF-BB used as a chemotacticant, PDGF-BB-induced VSMC migration was reduced to 66 and 60% by TUPS and 14,15-EET, respectively, at 6 h in human VSMCs (Fig. 4, C and D). Similar results were obtained in rat primary cultured VSMCs (data not shown). The effect of sEH may be dependent on levels of EETs; thus we measured the ratio of 14,15-DHET/EET with ultra-high-performance LC-MS/MS after treatment with PDGF in human VSMCs. Indeed, treatment with 20 ng/ml of PDGF for 24 h increased the ratio of EET to DHET, whereas TUPS reversed the effect of PDGF on EET degradation (Fig. 4E).

To further characterize sEH function in VSMCs, human and rat primary VSMCs were infected with Ad-GFP or Ad-sEH, and migration distance was investigated. In Ad-sEH-infected human VSMCs, the ratio of EET to DHET increased about onefold (Fig. 5A), and the mean migration distance was increased to 1.80-, 1.54-, and 1.50-fold compared with Ad-GFP-infected cells at 6, 12, and 24 h, respectively, after scratch wounding (Fig. 5B). Moreover, transwell assay revealed increased migration activity with Ad-sEH transfection at 6 h in humans (Fig. 5C). A similar trend was also observed in rat VSMCs (data not shown).

As the fundamental mechanism of VSMC migration, VSMCs undergo phenotypic switching in response to injury, along with loss of expression of VSMC differentiation markers (12). To gain insight into the effect of sEH on VSMC phenotype, we examined the expression of differentiation markers in VSMCs transfected with Ad-sEH. Overexpression of sEH switched VSMCs from a contractile to synthetic phenotype, as evidenced by an increased level of OPN and decreased level of α-SMA (Fig. 5, D–G).

Fig. 6. Neointima formation in injured carotid arteries attenuated by TUPS. Rats underwent carotid-artery balloon injury. A: representative H&E staining of carotid arteries with sham surgery (left) or day 3 (middle) and day 7 (right) after balloon injury (×100) (a–e) and higher amplification (×400) (f–j). B–E: quantification of neointima area, media area, ratio of neointima to media, and circumference of external elastic lamina (EEL) in H&E-stained sections. Data are means ± SD, n = 7–9 per group, *P < 0.05, **P < 0.01.
sEH inhibition attenuated neointima formation in injured carotid arteries. Migration of VSMCs plays a key role in the development of neointima thickening. We next examined whether sEH inhibition could reduce intimal hyperplasia in vessels after carotid-artery balloon injury in rats. Male rats received TUPS (0.65 mg·kg⁻¹·day⁻¹) or vehicle by drinking water for 7 days, followed by carotid-artery injury. As an sEH inhibitor, TUPS was able to dramatically reduce sEH activity in rats (4). H&E staining demonstrated that the neointima area of arteries was reduced to 65 and 44% by TUPS treatment on days 3 and 7, respectively (Fig. 6, A and B), whereas the media area was intact (Fig. 6C). Accordingly, the ratio of neointima to media area was lower with TUPS than vehicle treatment on both days 3 and 7 (Fig. 6D). However, the circumference of the external elastic lamina was comparable between the two treatments (Fig. 6E). Thus inactivation of sEH was sufficient to attenuate neointima formation after balloon injury. Immunofluorescence double staining for sEH and α-SMA showed significantly increased sEH protein expression in VSMCs in the media and neointima of injured arteries on day 3 compared with that of the sham-operated control group, which was decreased by TUPS (Fig. 7, A and B). However, the sEH level was not further increased on day 7, which implied that the upregulation of sEH happened at an early stage (Fig. 7B). In addition, the VSMCs had switched from a synthetic to a contractile phenotype by TUPS on days 3 and 7, as evidenced by decreased level of OPN (Fig. 7, C and D).

DISCUSSION AND CONCLUSIONS

sEH enzymatically hydrolyzes the conversion of EETs and other fatty acid epoxides to the less active corresponding diols (22). EETs, derived from arachidonic acid by epoxygenation through cytochrome-P450 mono-oxygenases, have multiple biological functions, but difficulties arise with long-term EET treatment because they are rapidly metabolized and incorporated into membranes. Therefore, considerable interest has arisen in developing methods to enhance the bioavailability of EETs by inhibiting sEH. As expected, inhibition of sEH prevents several cardiovascular diseases, including high blood pressure, cardiac fibrosis, inflammation, and atherosclerosis (16, 19, 24). Therefore, the enzyme activity of sEH appears to play an important role in the development of cardiovascular disease. However, evidence of sEH expression and its role in humans is lacking. In our study, we found that 1) sEH protein was highly expressed in VSMCs of human coronary atherosclerotic arteries; 2) PDGF-BB posttranscriptionally upregulated sEH expression in rat primary VSMCs by stabilizing sEH
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protein; 3) overexpression of sEH promoted the migration and phenotype switching in rat primary VSMCs, which could be reversed by the sEH inhibitor TUPS; and 4) sEH protein expression was increased in balloon-injured carotid arteries of rats, and TUPS attenuated the neointima formation.

Although sEH is expressed in many organs (8), sEH protein levels in human coronary atherosclerotic lesions have not been reported. We found more sEH-positive sections in plaques of patients with than without coronary atherosclerosis. Moreover, the sEH-positive cells were located in VSMC-marker-positive areas, which implied that the high expression of sEH may play a role in the development of atherosclerosis via VSMCs. Migration of VSMCs, which occurs under the influence of basic fibroblast growth factor and inflammatory factors, plays an essential role during the progression of cardiovascular diseases such as atherosclerosis and restenosis (11). sEH was previously reported to be involved in regulating VSMC proliferation (7, 14). We extended these findings and found that the sEH inhibitor TUPS reversed the migration of primary rat VSMCs induced by PDGF-BB. Moreover, the overexpression of sEH promoted the migration and phenotype switching of primary VSMCs. EETs were shown to possess antimigratory effects on VSMCs through the cAMP-protein kinase A pathway, which might explain the effect of sEH on VSMC migration (17). In our study, we found that PDGF-BB upregulated sEH and promoted EET degradation, which supported the role for EETs in VSMC migration.

sEH has many regulatory mechanisms at the transcription and protein levels. sEH expression can be regulated by 15d-PGJ2, homocysteine, angiotensin II, and tyrosine nitration, as well as by peroxisome proliferator-activated receptor-γ agonists (2, 5, 6, 18, 24). Our previous studies showed that sEH upregulation contributes to hypertension and cardiac hypertrophy (3, 23). In the present study, PDGF-BB induced sEH expression in dose- and time-dependent manners. However, our quantitative real-time RT-PCR and promoter deletion transfection data in rat primary VSMCs showed that PDGF-BB regulated sEH posttranscriptionally via stabilizing sEH protein.

Although inhibition of sEH prevents VSMC migration, the effects of sEH inhibition on vascular remodeling in vivo were controversial. In spontaneously hypertensive stroke-prone rats, sEH inhibition or Ehpx2 deficiency reduced the inward remodeling in a common carotid ligation model rather than wire-induced vascular injury in femoral arteries, which indicated the mechanisms are endothelium dependent (15). However, Ehpx2 deficiency was found to attenuate neointima formation in a femoral artery cuff model but not following carotid artery ligation in apoE−/− mice (14). In addition, sEH inhibitor is able to attenuate the development of atherosclerosis and abdominal aortic aneurysm formation but not ligation-induced carotid artery remodeling in apoE−/− mice with angiotensin II infusion (25). Those two studies suggested that the antivascular remodeling effect of sEH inhibitor and Ehpx2 deficiency in hyperlipidemia models is dependent on lowering lipid level. In our study, we performed the artery injury surgery in the carotid artery of rat with normal lipid level. The discrepancy between our study and that of Simpkins et al. (15) might be caused by species specificity.

Elucidating the mechanism controlling VSMC migration is critical for understanding the pathogenesis and progression of cardiovascular diseases. Our finding provides evidence that sEH is involved in the progression of neointima formation by promoting VSMC migration, which suggests that sEH inhibition may be a potential therapeutic target for cardiovascular diseases.

ACKNOWLEDGMENTS

Y. Zhu is an investigator in the Collaborative Innovation Center of Beijing for Cardiovascular Research. Present address of L. Huo: Department of Pathology, Guangdong Women and Children’s Hospital, Guangzhou Medical College, Guangzhou, China. Present address of H. Su: College of Forensic Medicine, Xi’an Jiaotong University Health Science Center, Xi’an, Shaanxi 710061, PR China.

GRANTS

This study was supported in part by Major National Basic Research Grant of China 2012CB517500 and by National Natural Science Foundation of China Grants 81121061, 81130002, and 81322006.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.Z. conception and design of research; Q.W., J.H., W.D., and H.S. performance of experiments; I.H. analyzed data; D.T. and B.H. interpreted results of experiments; Q.W. prepared figures; D.A. drafted manuscript; C.W., B.H., and D.A. edited and revised manuscript; Y.Z. and D.A. approved final submission.

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