Soluble epoxide hydrolase as a therapeutic target for cardiovascular diseases

John D. Imig and Bruce D. Hammock‡*

Abstract | The cardiovascular effects of epoxyeicosatrienoic acids (EETs) include vasodilation, antimigratory actions on vascular smooth muscle cells and anti-inflammatory actions. These endogenous lipid mediators are broken down into diols by soluble epoxide hydrolase (sEH), and so inhibiting this enzyme would be expected to enhance the beneficial cardiovascular properties of EETs. sEH inhibitors (sEHIs) that are based on 1,3-disubstituted urea have been rapidly developed, and have been shown to be antihypertensive and anti-inflammatory, and to protect the brain, heart and kidney from damage. Although challenges for the future exist — including improving the drug-like properties of sEHIs and finding better ways to target sEHIs to specific tissues — the recent initiation of the first clinical trials of sEHIs has highlighted the therapeutic potential of these agents.

Eicosanoids
Lipid mediators that are derived from the 20-carbon-atom arachidonic acid or a similar fatty acid.

Olefin bond
A double bond that links carbon atoms in an unsaturated hydrocarbon.

**Department of Pharmacology and Toxicology and the Cardiovascular Research Center, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226, USA.*
‡Department of Entomology and Cancer Center, University of California at Davis, One Shields Avenue, Davis, California 95616, USA.
e-mails: bdhammock@ucdavis.edu; jdimig@mcw.edu
doi:10.1038/nrd2875

Many of the enzymes, receptors and eicosanoid metabolites of the arachidonate cascade (FIG. 1) are key therapeutic targets, particularly for inflammatory disease. The first pathway to be targeted was the cyclooxygenase (COX) pathway, which produces prostaglandins. Indeed, aspirin and non-steroidal anti-inflammatory drugs (NSAIDs), including inhibitors of COX2 (also known as PTGS2), are effective drugs that treat pain and inflammation[1,2].

These drugs may also be useful for treating or preventing cardiovascular disease: it is thought that inhibition of blood clotting by aspirin can reduce the risk of ischaemic events such as heart attacks and stroke[1], and prostacyclin analogues are used for the treatment of pulmonary hypertension[3,4]. However, enthusiasm for targeting the COX pathway was diminished by the increased incidence of acute renal failure, myocardial infarction and thrombotic stroke in patients treated with COX2 inhibitors[1,2,5,6].

The generation of leukotrienes by lipoxygenase (LOX) was the second eicosanoid and inflammatory pathway to be therapeutically targeted. Arachidonate 5 lipoxygenase (ALOX5) and leukotriene receptor antagonists have been developed for the treatment of asthma and seasonal allergies[7,8]. These two eicosanoid pathways are becoming increasingly important therapeutic targets as novel receptors and metabolites are identified and their roles in many diseases are better defined.

A third eicosanoid pathway, that of cytochrome P450 (CYP), was first described in 1980. It comprises two enzymatic pathways[9,10,11], catalysed by the hydroxylases and the epoxygenases. The hydroxylase CYP enzymes convert arachidonic acid into hydroxyeicosatetraenoic acids (HETEs). 20-HETE, the main metabolite of this pathway, is pro-inflammatory and important to vascular function[12,13]. This pathway and metabolite are currently being targeted for the treatment of cardiovascular diseases such as hypertension and stroke[13–16]. The epoxygenase CYP enzymes generate epoxyeicosatrienoic acids (EETs), by catalysing the epoxidation of arachidonic acid olefin bonds, resulting in the production of four regioisomeric EETs: 5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET. EETs are endothelium-derived hyperpolarizing factors (EDHFs) that protect from ischaemic injury and have anti-inflammatory actions in canine and rodent disease models[17–21].

Conversion of EETs to their corresponding diols (dihydroxyeicosatrienoic acids; DHETs) by soluble epoxide hydrolase (sEH) is responsible for decreasing EET levels and thereby diminishing their beneficial cardiovascular properties[20,21]. Inhibition of this enzyme is therefore a promising therapeutic strategy for cardiovascular disease. Recently, sEH inhibitors (sEHIs) have been developed to enhance the cardiovascular actions of EETs. This article highlights the development of sEHIs as cardiovascular therapeutics and discusses the potential of this treatment and challenges that lie ahead.

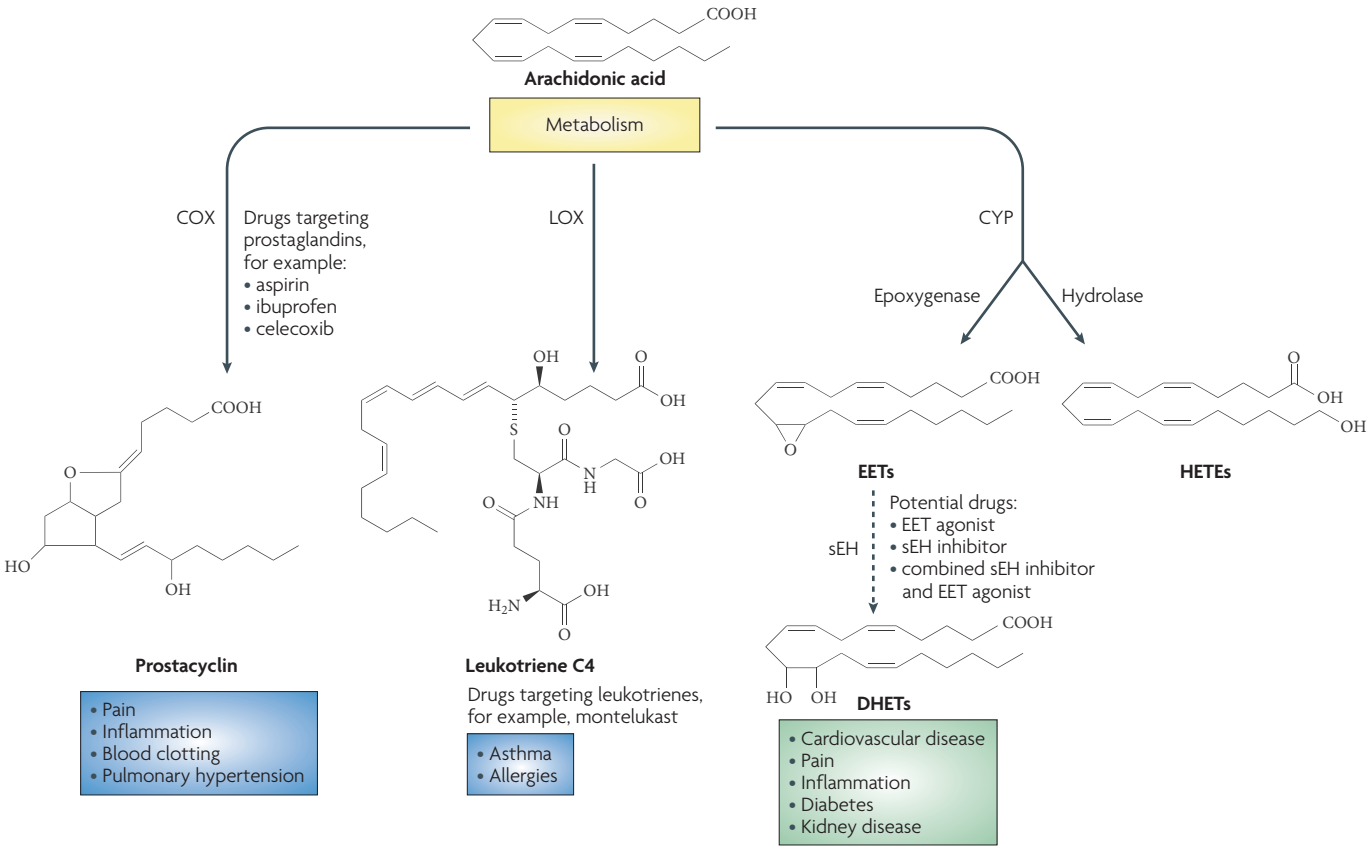

Figure 1 | **Therapeutic targets in the arachidonate cascade.** Three key pathways — the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) pathways — can metabolize arachidonic acid. Inhibitors of COX1 (also known as PTGS1) and COX2 (also known as PTGS2) are used for the treatment of pain, inflammation and blood clotting, and prostacyclin analogues are used to treat pulmonary hypertension. Leukotriene receptor antagonists that inhibit the cysteinyl leukotriene CysLT1 receptor are used to treat asthma and allergies. Soluble epoxide hydrolase (sEH) inhibitors that increase epoxyeicosatrienoic acid (EET) levels are being developed for the treatment of cardiovascular diseases and inflammation. DHETs, dihydroxyeicosatrienoic acids; HETEs, hydroxyeicosatetraenoic acids.

Biological aspects of EETs

Since the first descriptions of the biological actions of EETs, which included increases in epithelial transport in the kidney and dilation of small mesenteric resistance arteries, there has been growing interest in these eicosanoid metabolites[22,23]. Interest in EETs was greatly increased in 1996 after the identification of EETs as EDHFs[17]. Over the past decade, it has become increasingly apparent that EETs have many cardiovascular actions, most of which seem to be cardiovascular protective.

The cellular signalling mechanisms that are responsible for the biological actions of EETs continue to be intensively investigated. There is ample evidence that EETs bind to receptors that are coupled by a G protein to intracellular signalling cascades[24,25]; however, an EET receptor has yet to be identified. EETs could also function inside the cell by coupling to and activating ion channels, signalling proteins or transcription factors. Experimental evidence supports an intracellular mechanism of action, in that EETs are incorporated into cell membrane phospholipids, and bind to fatty-acid-binding proteins and peroxisome proliferator-activated receptor-γ (PPARγ)[21,24,26,27]. The biological activities and cellular signalling mechanisms of EETs have been comprehensively reviewed elsewhere[28,29].

As with other eicosanoid pathways, the cellular signalling mechanisms and biological activities of EETs vary depending on the cell type and tissue. Other experimental issues have made it difficult to investigate EETs and the CYP enzymatic pathways. The common concerns relate to the quality and purity of regioisomeric EETs and the correct method of using them. Similarly, investigations in cell culture systems are limited by the fact that the levels of epoxygenase and epoxide hydrolase enzymes decrease rapidly following cell isolation. Experimental approaches to circumvent these issues include the generation of genetically manipulated mice, transfection of cell culture lines with CYP enzymes, and the development of EET analogues and antagonists that have improved chemical properties and greater stability[18,30]. Moreover, EET receptor identification could help to clarify the apparent biological heterogeneity of EET signalling, much like the discovery of multiple prostaglandin E2 (PGE2) receptors helped explain away the

Endothelium-derived hyperpolarizing factor
A substance released by endothelial cells that hyperpolarizes vascular smooth muscle cells, resulting in vasodilation.

apparent contradictions in biological and cell signalling mechanisms of PGE2 (REFS 31,32). Despite these experimental concerns, there has been tremendous progress in determining EET biological actions, and EETs remain an attractive therapeutic target for cardiovascular diseases.

Vascular actions of EETs. The roles of EETs as vasodilators and EDHFs are the most extensively examined cardiovascular actions of these signalling molecules. Vasodilation in response to EETs has been observed in numerous organs, including the heart, brain, kidney, skeletal muscle and intestine[13,17,23,33,34]. By contrast, EETs cause vasoconstriction in the lung — a finding that was not unexpected because the effects of prostaglandins in this vasculature are opposite to those in other organs[35,36].

All regioisomeric EETs are vasodilators, with 11,12-EET and 14,15-EET consistently exhibiting greatest vasodilator activity[21,34]. These two regioisomeric EETs are generated by endothelial cells and dilate blood vessels by activating large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels on vascular smooth muscle cells[33,37,38,39], resulting in K^+ efflux from the smooth muscle cell and subsequent membrane hyperpolarization[17,38]. There is evidence that EET activation of K_{Ca} channels on vascular smooth muscle cells involves cyclic AMP activation of protein kinase A (PKA) and ADP ribosylation of the α-subunit of the stimulatory G protein ($G_s\alpha$)[39–42]. The ability of EETs to activate K_{Ca} channels and dilate blood vessels can be regulated by sEH-mediated conversion to DHETs, which have little or no ability to cause vasorelaxation[34,38,43]. Therefore, sEH inhibition improves dilator activity in human blood vessels, by impeding the conversion of EETs to DHETs[43]. EETs or sEHIs oppose the vasoconstrictor activities of the pro-hypertensive hormones endothelin 1 and angiotensin II (REF. 20). Therefore, decreased endothelial EET conversion to DHETs could be one mechanism responsible for the antihypertensive actions of sEHIs, as well as for their other cardioprotective properties.

Vascular homeostasis is controlled by endothelial cell and vascular smooth muscle cell proliferation and migration, and EETs and sEH seem to be important regulators of these cellular processes[18,30,44–49]. EETs promote angiogenesis and endothelial cell proliferation and migration. It has been shown that the epoxides, and not the corresponding diols, caused the proliferative effects[45]. In murine and human cell lines, EETs or overexpression of CYP2C epoxygenases lead to proliferative responses[30,46], which have been attributed to activation of two cell signalling pathways: the p38 mitogen-activated protein kinase (MAPK) pathway and the phosphatidylinositol 3-kinase–AKT (PI3K–AKT) pathway[30]. 11,12-EET activates MAPK, which upregulates cyclin D and AKT. AKT then phosphorylates forkhead factors and decreases the expression of the cyclin-dependent kinase inhibitor $p27^{kip1}$ in endothelial cells[46,47]. More recently, 11,12-EET-mediated proliferation, migration and tube formation in human umbilical vein cells was shown to be dependent on activation of sphingosine kinase 1, which phosphorylates sphingosine to generate spingosine-1-phosphate (S1P)[49]. By contrast, EETs have antimigratory actions in vascular smooth muscle cells. 11,12-EET and 14,15-EET moderately attenuated the migration of aortic smooth muscle cells in response to platelet-derived growth factor[50], and overexpression of CYP2J epoxygenase or inhibition of sEH also reduced smooth muscle cell proliferation and migration[50]. Activation of the cAMP-dependent PKA pathway and decreased cyclin D levels have been implicated in the antimigratory actions of EETs and sEHIs in vascular smooth muscle cells[30]. Although these findings suggest an effect on vascular smooth muscle cells by EETs and sEHIs, other reports have failed to show that EETs or sEHIs cause vascular smooth muscle proliferation[51,52]. More importantly, *in vivo* angiogenesis is stimulated by EETs in a subcutaneous-sponge model, and inhibition of sEH enhanced these pro-angiogenic and neovascularization responses[48]. The effects of EETs and sEHIs on the proliferation and migration of endothelial and vascular smooth muscle cells highlight the possible importance of targeting this pathway in angiogenesis, atherosclerosis and other cardiovascular diseases.

Anti-inflammatory actions of EETs. Inflammation and inflammatory diseases contribute substantially to vascular and end-organ damage and cardiovascular disease progression[53,54]. Similarly, interactions between inflammation and the epoxygenase pathway, which can affect cardiovascular function in disease states, have been clearly established[55–58]. Cytokines can decrease CYP2C expression and oppose epoxygenase-mediated vasodilation[18,59]. Conversely, inhibition of tumour necrosis factor (TNF) or CC-chemokine receptor 2 result in an increase in kidney CYP2C expression, and decrease renal injury in hypertension[55,56].

Experimental evidence suggests that EETs interfere with activation of the transcription factor nuclear factor κB (NF-κB) to exert their vascular anti-inflammatory effects[57,58]. 11,12-EET, but not other regioisomeric EETs, prevented TNF-induced activation of NF-κB and increased the expression of vascular cell adhesion molecule 1 (VCAM1) in endothelial cells[57]. Similarly, CYP2J epoxygenase overexpression in endothelial cells decreased NF-κB activation[57].

Although additional studies are required to determine the exact cellular signalling mechanisms responsible for the anti-inflammatory actions of EETs, there is considerable evidence that EETs decrease inflammation. Other anti-inflammatory actions that are attributed to EETs include decreased aggregation of human polymorphonuclear leukocytes and decreased leukocyte adhesion to endothelial cells[58–61]. EETs also decreased interleukin-1β-induced fever. In this case, 11,12-EET (administered to the brain) had a greater antipyretic action than other EETs[62,63]. Studies using sEHIs also support the notion that EETs have anti-inflammatory actions[64–67]. Inhibition of sEH decreased cigarette smoke-induced lung inflammation and significantly reduced the numbers of neutrophils, alveolar macrophages and lymphocytes in the bronchial fluid[68]. These findings suggest that sEHIs could be protective against the

Angiogenesis
The formation of new blood vessels.

End-organ damage
Injury to major organs, particularly the heart, brain and kidneys, owing to disease.

Cytokine
A regulatory protein released by immune cells that acts as a mediator in the generation of the immune response.

deleterious effects of inflammation associated with cardiovascular diseases, and that they could provide treatment for other inflammatory diseases.

Epoxygenase pathway polymorphisms in human disease. The notion that both the EETs and sEH are therapeutic targets for human disease is supported by genetic studies. There are a number of polymorphisms in the gene encoding sEH (*EPHX2*) that result in amino-acid substitutions that influence sEH enzymatic activity[69–73]. Two studies have linked genetic variation in *EPHX2* to an increased risk of coronary artery disease, and a third study found that smoking further increased the risk associated with this genetic variation[69,72,74]. Other cardiovascular diseases that are associated with genetic variation in *EPHX2* include ischaemic stroke and hypercholesterolaemia[71,75]. Variations in the genes encoding epoxygenases CYP2J2, CYP2C8 and CYP2C9 can affect transcription or enzymatic activity[73,76,77]. These CYP2C8 and CYP2C9 variants have been associated with myocardial infarction and cardiovascular disease[76,77]. Taken together, these findings in the patient population provide evidence that sEHIs could have potential therapeutic value in a wide range of cardiovascular diseases and that they may be of particular benefit for patients of certain genotypes.

Design of sEHIs

The rapid development of sEHIs for *in vivo* use and clinical testing in the past decade is remarkable. A landmark study published in 2000 (REF. 78) showed that injection of a sEHI to the spontaneously hypertensive rat (SHR) lowered blood pressure. This study was followed up by the first demonstration that chronic inhibition of sEH lowered blood pressure in angiotensin-induced hypertension[79]. Another breakthrough came in 2005, when it was shown that an orally administered sEHI was antihypertensive and slowed the progression of renal damage[80]. Following this, a number of studies have provided exciting findings on the broad potential for sEHIs as cardiovascular therapeutic agents, and a first in class sEHI began clinical Phase IIa testing this year (Arete Therapeutics initiates Phase IIa clinical trial for AR9281, a novel sEH inhibitor to treat type 2 diabetes; see Further information). In this section, we describe the evolution of selective sEHIs, from enzyme inhibition *in vitro* to oral administration in rodents and subsequently humans.

There are two well studied α–β-hydrolase fold epoxide hydrolase enzymes that differ by subcellular localization and substrate selectivity[56,81,82]. The microsomal epoxide hydrolase (mEH) is involved in the metabolism of environmental contaminants, and it has been studied extensively in this role[81,82]. The sEH was first discovered in studies on the metabolism of a terpenoid epoxide that mimicked insect juvenile hormone[81,82]. At the same time, EETs were being established as endogenous lipid mediators with biological activity. Subsequently, it was discovered that arachidonic acid and linoleic acid epoxides are metabolized by sEH, and sEH coverts these epoxides to diols with high V_{max} and low K_m[81,82,83].

More recently, the sEH gene and transcript have been cloned and the sEH structure and catalytic mechanism determined. The mammalian sEH is a homodimer with monomers arranged in an anti-parallel form[81,82,83]. Each monomer is composed of two domains: the carboxy-terminal domain, which has epoxide hydrolase activity, and the amino-terminal domain, which hydrolyses phosphates on lipophilic backbones[84,85,86]. This highly conserved enzyme is widely distributed in tissues, including the liver and kidney, in which sEH-specific activity is highest[85,87,88,89].

The functional importance of the N-terminal domain of the mammalian sEH remains unclear. It has phosphatase activities that dephosphorylate polyisoprenyl phosphates, which are known to regulate cholesterol levels[84,86,90]. It has been suggested that the N-terminal domain could stabilize the epoxide hydrolase activity, because expression of the human sEH C-terminal domain alone has reduced activity[90]. The N-terminal domain might promote dimerization of the sEH enzyme[89,91]. Current sEHIs inhibit the epoxide hydrolase activity of the C-terminal domain without affecting the phosphatase activity of the N-terminal domain[85]. Selective inhibitors of the N-terminal domain, which would be useful for determining the functions of this domain, have yet to be developed.

The first-generation sEHIs were potent competitive inhibitors and included chalcone oxides and glycidols[21,85,92]. Unfortunately, these alternative substrates are rapidly inactivated by glutathione and glutathione transferases, making them difficult to use in tissue samples and *in vivo*[21,85]. A breakthrough came when amides, ureas and carbamates were found to be potent and stable transition state inhibitors of sEH, because these tools facilitated experiments to investigate the endogenous roles of this enzyme[85,93].

The design of these transition state mimics was based on the knowledge of the catalytic mechanism of the enzyme[85,93]. X-ray structures of the murine and human enzyme, modelled with these urea sEHIs, suggested that the urea is the central pharmacophore and that hydrogen bond-stabilized salt bridges were formed between the urea moiety and residues of the C-terminal sEH active site[85,93] (FIG. 2A). This supports the hypothesis that ureas imitate features that are present in transient intermediates or transition states that occur during opening of the epoxide ring by the sEH. These 1,3-disubstituted ureas, carbamates and amides inhibit the C-terminal epoxide hydrolase activity of the sEH enzyme with nanomolar K_i values but do not substantially alter the phosphatase activity of the N-terminal domain[85,93]. The urea pharmacophore seemed to be the most potent inhibitor. However, with suitable substituents, amides and carbamates of equal potencies can be obtained. Subsequent modifications to improve *in vivo* stability of sEH allowed evaluation of the role of this enzyme in cardiovascular diseases[65,85,93,94].

Although the mEH has the same catalytic mechanism as the sEH, it is possible to design inhibitors with more than 1000-fold selectivity for one hydrolase over the other, using specific substituents on the amides and

Insect juvenile hormone
A hormone in arthropod larvae that inhibits the enzyme ecdysone, thereby preventing moulting and the development of larvae into adults.

V_{max}
The velocity of an enzyme-catalysed reaction at infinite concentration of substrate.

K_m
The substrate concentration at which the enzyme-catalysed reaction rate is half V_{max}.

Transition state inhibitor
A species that resembles the transition complex formed in the catalytic cycle — the state in which the enzyme has maximum free energy.

K_i
The equilibrium dissociation constant for an inhibitor and a specific enzyme target. It is the concentration of inhibitor that is required to decrease the rate of the reaction to half of the maximum value.

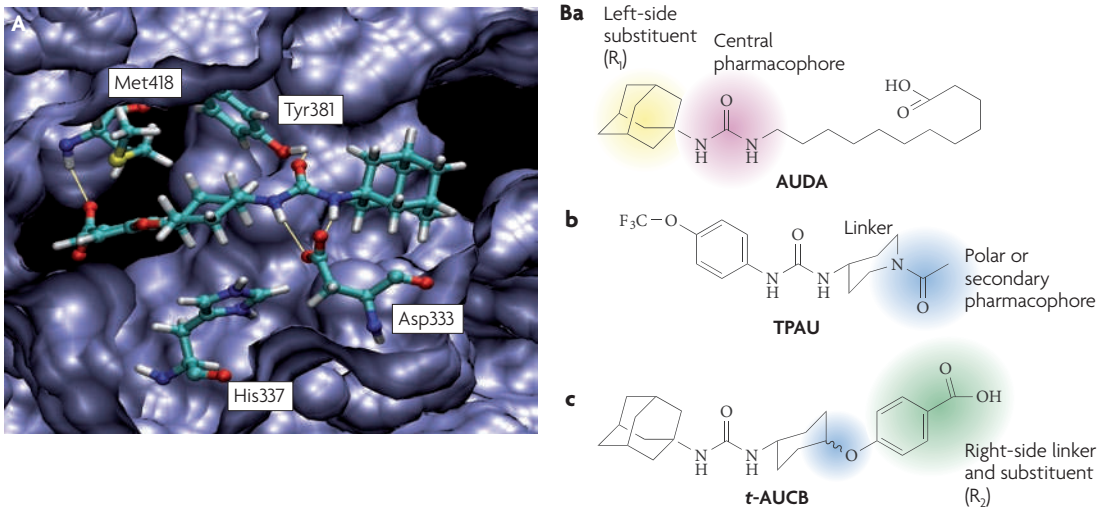

Figure 2 | **Soluble epoxide hydrolase inhibitor (sEHI) structures and binding to the enzymatic pocket. A** | The structure of the sEH enzymatic pocket with bound sEHI, *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB). The residue Tyr465 is omitted for clarity. The structure was prepared using Scigress Explorer Standard version 7.7.0.49, the atomic coordinates of the human sEH were retrieved from the Protein Data Bank (PDB number 1VJ5) and the image was produced using freewares VMD 1.8.6 and POV-Ray 3.6. The key amino-acid residues that form the binding site for the sEHI are shown. **Ba** | The compound 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) contains the central pharmacophore that forms multiple hydrogen bonds in the enzyme catalytic site. Urea, amide and carbamate substituents have been used for the central pharmacophore. The R_1 or left side of the molecule rests in a hydrophobic pocket of the sEH catalytic tunnel. The hydrophobic right side of AUDA was designed to mimic 14,15-epoxyeicosatrienoic acid (EET). AUDA is a highly potent sEHI but must be formulated carefully for use *in vivo*. **Bb** | 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl)urea (TPAU) is a potent sEHI, illustrating that a polar secondary pharmacophore 7–8 Å from the central pharmacophore increases solubility while maintaining potency. It has a piperidine linker group between the central pharmacophore and secondary pharmacophore. **Bc** | *t*-AUCB has an ether as the secondary pharmacophore, with an R_2 on the right side reaching towards the enzyme surface and mimicking the carboxylate of EETs. The *cis* isomer (not shown) is also an active sEHI. Both TPAU and *t*-AUCB are highly potent and have good oral availability and pharmacokinetic characteristics. *t*-AUCB is more broadly active across multiple species. The image shown in part **A** is courtesy of S. H. Hwang, University of California at Davis, USA.

ureas[85,95]. The sEHIs tested seem to be highly selective for the sEH, and the more than 300 positive hits from a National Institutes of Health screen of sEHs do not consistently inhibit other enzymes[96]. The anticancer drug sorafenib (Nexavar; Bayer/Onyx) — a potent inhibitor of several kinases — is also a potent sEH inhibitor. This joint inhibition seems to be limited to sEHIs that have a closely related chemical structure to sorafenib. It is possible that the sEH inhibition by sorafenib reduces some of the side effects that are associated with this drug class when drugs in this class are used at high doses.

The first report to show *in vivo* biological effects of a sEHI used a single bolus dose of *N,N'*-dicyclohexylurea, which lowered blood pressure in hypertensive rats[78]. Chronic administration was first achieved with 1-cyclohexyl-3-dodecyl-urea (CDU), which had antihypertensive actions when injected intraperitoneally for 4 days[79].

These large-molecular-mass ureas have limited solubility in both water and organic solvents, and so careful formulation is needed to show *in vivo* efficacy[81,85,98]. Incorporation of functional polar groups into one of the alkyl chains of 1,3-disubstituted urea sEHIs resulted in compounds that were weak structural mimics of EETs with improved physical properties[94,99]. One example was 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) (FIG. 2B), which has been widely used in cultured cells and animals[21,65,81,85]. Although AUDA can be orally administered, it requires dimethyl sulphoxide (DMSO) for *in vitro* experiments, and a considerable amount of 2-hydroxylpropyl β-cyclodextrin for it to be administered in drinking water for *in vivo* studies[21,65,81]. If lipophilic compounds do not remain in solution, bioavailability decreases dramatically. As expected for an enzyme with a largely hydrophobic catalytic tunnel, addition of polar groups in general results in a substantial reduction in potency. However, the addition of a polar group — termed a secondary pharmacophore — such as an ether, ester, amide, sulphonamide, alcohol or ketone approximately 7–8 Å from the polar group of the central pharmacophore increased the water solubility of the inhibitor without reducing potency[81,94,98,100]. The application of this concept was used to produce other drug-like sEHI molecules, including *trans*-4-[4-(3-adamantan-1-yl-ureido)-cyclohexyloxy]-benzoic acid (*t*-AUCB), 1-trifluoromethoxyphenyl-3-(1-acetylpiperidin-4-yl) urea (TPAU) (FIG. 2B), and others that have both excellent potency and efficacy in many species.

In parallel to the improvement of sEHIs for experimental studies, the development of sEHIs for use in humans has advanced. These sEHIs are initially being developed for the treatment of hypertension.

Catalytic tunnel
The space within the enzyme to which the substrate binds for catalysis.

Arête Therapeutics began Phase I clinical trials in healthy volunteers with the first in class sEHI, AR9281, in October 2007, and Phase II trials are in progress for the treatment of hypertension and type 2 diabetes (Arete Therapeutics initiates Phase IIa clinical trial for AR9281, a novel sEH inhibitor to treat type 2 diabetes; see Further information). In less than a decade, sEH has gone from obscurity to a recognized therapeutic target, and sEHIs have progressed from their first demonstration of anti-hypertensive actions to being tested for the treatment of diseases in humans.

Cardiovascular therapeutic effects of sEHIs

sEHIs have cardiovascular-protective effects in hypertension, cerebral ischaemia, cardiac ischaemia, cardiac hypertrophy and atherosclerosis[79,101–105], suggesting that these agents have broad potential for the treatment of many cardiovascular diseases and associated morbidity[21,65,106]. The progression of end-organ damage, inflammation and endothelial dysfunction that are associated with cardiovascular disease are also attenuated by sEH inhibition[67,103,104,107]. Studies conducted in mice with *Ephx2* gene deficiency suggest that the effects of sEHIs are due to inhibition of the C-terminal epoxide hydrolase domain[35,101,103,104,108–110]. Although studies on these *Ephx2*$^{-/-}$ mice have the potential to reveal the function of the N-terminal domain of the sEH enzyme, a role for this domain has remained elusive.

Antihypertensive effects. sEHIs have hypertensive effects in numerous animal models of hypertension[20,21,65] (FIG. 3). In the SHR, the urea *N,N'*-dicyclohexylurea lowered blood pressure and decreased urinary DHET excretion[78], and CDU (given once daily) lowered blood pressure in hypertension driven by angiotensin infusion in the rat[79]. The first sEHI to be successfully administered orally to hypertensive animals[80], AUDA, lowered blood pressure in rat and mouse models of hypertension[80,102,111]. Blood pressure was consistently lowered by 25–30 mmHg in the rat models of hypertension, and to a greater extent in mice that had angiotensin-dependent hypertension[80,102,111]. The mechanism by which AUDA lowers blood pressure seems to be dependent on decreased vascular resistance and enhanced Na^+ excretion by the kidney[67,80,102]. These findings are consistent with the biological actions of EETs to dilate blood vessels and inhibit renal tubular Na^+ reabsorption[20,21,22,112]. It was also in these initial hypertension studies that the first evidence for end-organ protection by sEHIs was recognized[65,67].

There have been some conflicting reports on sEHI-mediated lowering of blood pressure in rats and mice. These species differences may be because many tissues in some rat strains, including liver and kidney, have low sEH activity, and sEH levels between rat strains vary dramatically. Although the first demonstration of the ability of sEHIs to lower blood pressure was in the SHR, subsequent studies have shown variable levels of blood pressure-lowering in this model[113–115], which could be due in part to polymorphisms in the *Ephx2* gene between SHR strains[113,116]. There are also conflicting results on sEHI-mediated changes in blood pressure in *Ephx2*$^{-/-}$ mice. The initial *Ephx2*$^{-/-}$ male mice had decreased blood pressure that could not be confirmed when these mice were back-bred into a C57/BL6 background or in an independently generated *Ephx2*$^{-/-}$ mouse colony[117,118]. More recently, studies in a second *Ephx2*$^{-/-}$ C57/BL6 back-bred colony did not show lower blood pressure in males at baseline, but deoxycorticosterone (DOCA)-salt-induced hypertension was attenuated[119]. Interestingly, each *Ephx2*$^{-/-}$ mouse colony had higher levels of EET and 20-HETE than controls, which could have offset some of the blood pressure effects[118]. Although the antihypertensive actions of sEHIs have been variable, the ability of sEHIs to protect from end-organ damage associated with cardiovascular diseases has been much more consistent.

Kidney-protective properties. Chronic sEHI treatment attenuated renal vascular and glomerular injury in rats with angiotensin-induced hypertension, showing that sEHIs provided protection from end-organ damage associated with cardiovascular disease[67,80]. In this model, sEHIs produced a decrease in collagen expression in glomeruli and tubular cells, as well as decreasing vascular hypertrophy. Moreover, urinary albumin excretion was decreased and macrophage infiltration was reduced. These studies also showed that starting sEHI treatment either at the onset or after the establishment of hypertension provided similar protection to the kidney as when treatment was begun at an earlier stage.

Although the renal protection afforded by sEHIs in these animal models of hypertension could have been a result of the decrease in blood pressure, a more recent study in diabetic Goto–Kakizaki rats clearly showed that AUDA provides renal protection independently of lowering blood pressure[107]. Moreover, the elevated plasma cholesterol and triglyceride levels that are observed in theses rats were not lowered by AUDA treatment[107]. In addition to the studies in animal models of chronic progressive kidney disease, studies in mice have shown that sEHIs can provide protection from acute renal injury that is induced by the chemotherapeutic agent cisplatin[120]. Inhibition of sEH decreased blood urea nitrogen levels for up to 96 hours and reduced the tubular damage that is associated with cisplatin[120]. Overall, studies have consistently found improved renal vascular function, decreased glomerular injury and a decrease in renal inflammation, which highlight the promise of sEHIs as a treatment for acute and chronic kidney disease.

Cardiac-protective properties. The cardiac-protective properties of sEHIs provide a key source of therapeutic potential, especially in protecting from myocardium ischaemic events. *Ephx2*-deficient mice have improved recovery of left ventricular developed pressure and reduced infarct size following ischaemia and reperfusion, and are also protected from developing pressure overload-induced heart failure and cardiac arrhythmias[104]. The ability of sEHIs to improve cardiac function has been established in various experimental models and species[19,104,108,121]. AUDA reduces the cardiac infarct size in dogs, which is similar to the effect observed with 14,15-EET administration[19]. Similar effects were

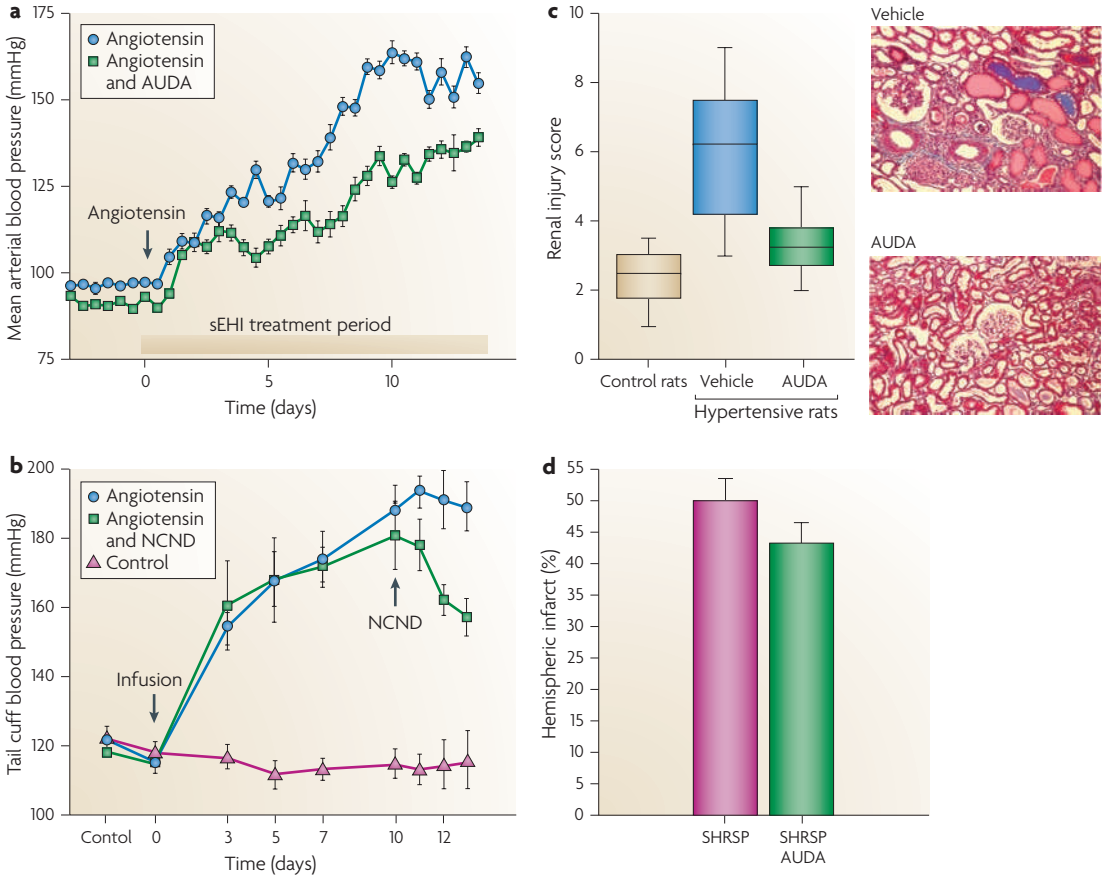

Figure 3 | **Antihypertensive and end-organ protective actions of soluble epoxide hydrolase inhibitors (sEHIs).** **a** | The increase in blood pressure following angiotensin infusion is decreased by oral administration of the sEHI 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA) at the onset of hypertension. **b** | Administration of the sEHI *N*-cyclohexyl-*N*-dodecyl urea (NCND) after the development of hypertension that is induced by angiotensin infusion lowers blood pressure. **c** | sEHI treatment decreases renal injury in diabetic hypertensive Goto–Kakizaki rats independently of lowering blood pressure. Tissue sections from vehicle-treated rats show renal injury, including fibrinoid necrosis, hyaline arteriopathy, tubular dilation and atrophy, and cast formation, which is decreased in AUDA-treated rats. **d** | sEHI decreases brain injury that is associated with cerebral ischaemia in spontaneously hypertensive rats that are stroke prone (SHRSP) independently of lowering blood pressure. Part **a** is modified, with permission, from REF. 80 © American Heart Association (2005). Part **b** is modified, with permission, from REF. 79 © American Heart Association (2002). Part **c** is modified, with permission, from REF 107 © Portland Press (2009). Part **d** is modified, with permission from REF. 115 © American Society for Investigative Pathology (2009).

seen in mice that were administered AUDA butyl ester (AUDA-BE) and subjected to left coronary artery occlusion followed by reperfusion[104]. Furthermore, in dogs and mice, the EET antagonist 14,15-epoxyeicosa-5(*Z*)-enoic acid (14,15-EEZE) inhibits the cardiac-protective effects of sEHIs[19,104]. Acute myocardial infarction or hypertension can result in cardiac hypertrophy, owing to ventricular remodelling[101,104,111,121]. The first evidence that sEHIs could attenuate cardiovascular hypertrophy was the observation that heart weight and collagen levels were decreased in rats with DOCA-salt-induced hypertension that were treated with a sEHI[111]. Similarly, cardiac hypertrophy in stroke-prone SHRs and angiotensin-infused rats was prevented by inhibition of sEH[101,122]. The cardiac-protective actions of sEHIs have also been found in mice with pressure overload-induced myocardial hypertrophy, in which sEHIs prevented the development of or reversed left ventricular hypertrophy[104,121]. This effect was linked to the ability of sEHIs to block NF-κB activation[121]. Although there is overwhelming evidence that *Ephx2* deficiency and sEHIs provide cardiac protection, *Ephx2*-knockout mice had reduced survival from cardiac arrest when cardiopulmonary resuscitation was performed[123]. Further experimental evidence is required to determine the potential for sEHIs to be used as therapies for various heart diseases.

Protection against ischaemic stroke and vascular disease. Another potential therapeutic use for sEHIs is protection from ischaemic brain damage that accompanies stroke. Chronic treatment with AUDA to stroke-prone SHRs decreases cerebral infarct size after middle cerebral artery occlusion[103,114,115,124]. Interestingly, blood pressure was not lowered in these hypertensive rats, supporting the notion that the cerebral-protective effects were independent of blood pressure[114]. *Ephx2*-deficient mice have

decreased infarct size following a cerebral ischaemia[103,124]. Ischaemic stroke protection has also been determined in a mouse model of focal ischaemia–reperfusion injury, in which administration of AUDA-BE or exogenous EETs resulted in at least a 50% reduction in infarct volume[124,125]. Moreover, administration of sEHIs 1 hour before the onset or at the start of reperfusion provided cerebral protection[124,125].

The mechanisms by which sEHIs protect the brain from ischaemic damage seem to be multi-modal and involve the cerebral vasculature and neurons[124,125]. EETs and sEHIs can protect neurons through anti-apoptotic and anti-inflammatory actions, and vasodilatory EETs regulate cerebral blood flow and could contribute to brain protection[124,125]. Angiogenic and attenuated vascular remodelling that allow for enhanced perfusion of the ischaemic area have been observed in the stroke-prone SHR treated with sEHIs[115]. However, these vascular changes do not occur in normotensive animals that also demonstrate decreased infarct volume when treated with sEHIs[115]. Taken together, these findings indicate that sEHIs have broad pharmacological potential for treating ischaemic stroke.

Other areas that are beginning to be explored include the effects of sEHIs on vascular remodelling, angiogenesis, diabetes and atherosclerosis. Inhibition of sEH decreased vascular hypertrophy in hypertension and decreased vascular smooth muscle cell proliferation in rats and cultured human cells[51,52,67]. In mice, angiogenic actions of EETs have been shown that were enhanced in the presence of a sEHI[48]. Increased microvascular densities and increased middle cerebral artery compliance were associated with AUDA treatment in the stroke-prone SHR[114,115]. More recent studies have shown that sEHIs or *Ephx2* deletion antagonizes neointimal formation *in vivo* by mechanisms that are endothelium dependent[105,126]. Atherosclerosis in apolipoprotein E-knockout mice was also reduced by sEHI treatment[105]. Thus, sEHIs may have therapeutic potential for specific types of vascular remodelling and atherosclerosis.

Anti-inflammatory properties. The anti-inflammatory actions of sEHIs are crucial to their end-organ-protective effects in cardiovascular disease models[18,65]. There is also strong evidence that sEHIs could be useful for treating inflammatory diseases[64,66,109,127]. AUDA-BE reduced the production of cytokines and pro-inflammatory lipid mediators and diminished lipopolysaccharide-induced mortality in mice[127]. Furthermore, topical application of sEHIs reduced lipopolysaccharide-induced thermal hyperalgesia and mechanical allodynia inflammatory pain in rats[66]. Although there is ample evidence that inhibition of sEH is anti-inflammatory, lipopolysaccharide-induced expression of inflammatory genes or neutrophil accumulation in the liver were not reduced in *Ephx2*-deficient mice or wild-type mice that were administered a sEHI[109].

Lung inflammation is another area in which sEHIs could have therapeutic value. In mice exposed to tobacco smoke, three aortic endothelial cell genes showed a threefold or greater increase in expression, one of which was *Ephx2* (REF. 68). Inhibition of sEH reduced macrophage infiltration into the rat lung exposed to tobacco smoke, and was further reduced by the combination of AUDA-BE and EET treatment[68]. Overall, these experimental findings reveal that sEHIs provide beneficial anti-inflammatory and analgesic actions.

Therapeutic potential and challenges

On the basis of studies in various animal models of cardiovascular disease, considerable interest has arisen in the therapeutic potential of sEHIs. Human studies are also providing evidence for a contribution of sEH to cardiovascular diseases and other disease states. One consideration is that the long-term treatment of many cardiovascular diseases requires an exceptionally good drug safety profile. To date, the large therapeutic index of sEHIs makes this class of compound particularly attractive in this respect. Because there are numerous accepted treatments for cardiovascular diseases such as hypertension, the requirement to perform clinical trials for a new class of drug and the high bar for safety for new therapies makes the route to the clinic expensive. It could be that other disease indications will be more attractive goals for the first sEHI Phase III trials. The findings from animal studies and the initial clinical trials will undoubtedly be expanded on in the future, and clinical translational studies will ultimately determine the best therapeutic uses and limitations for sEHIs. What are these potential therapeutic applications and what challenges lie ahead?

Outlook for novel therapeutic applications. The therapeutic potential of sEHIs for treating cardiovascular diseases seems to be promising. Patients with cardiovascular diseases are often treated over long periods with multiple medications for conditions such as high blood pressure, hypercholesterolaemia, high blood glucose levels and hyperlipidaemia, as well as several others. There is increasing evidence that sEHIs can synergize with existing medications and could be designed as combinational drugs[27,64,128]. Levels of COX2 protein are decreased by sEHIs, resulting in decreased PGE2 levels while maintaining the PGI2/thromboxane A2 ratio (PGI2/TXA2 ratio). This suggests that low-dose COX2 inhibitors and sEHIs in combination have additive or synergistic antihyperalgesic and anti-inflammatory effects without causing a decrease in the cardiotoxic PGI2/TXA2 ratio[64]. The complexity of the arachidonate cascade suggests that sEHIs will have interactions with other NSAIDs and inhibitors of ALOX5 receptors and leukotriene receptors that are currently on the market, which could be beneficial or detrimental as therapies depending on the other agents with which the sEHIs are combined. Further evaluation of sEHIs and their interactions with other eicosanoid pathways may highlight other mechanisms that have the potential to improve cardiovascular therapeutics.

Another interesting finding has been that AUDA has weak PPARα agonistic activity, suggesting the possibility of combination drugs based on this sEHI[128] that could be beneficial for patients with hyperlipidaemia and hypertension. Although AUDA failed to lower blood pressure,

Hyperalgesia
An abnormally increased sensitivity to painful stimuli.

Allodynia
An abnormal pain state, in which normally non-painful stimuli evoke pain responses.

Therapeutic index
(Also known as the therapeutic ratio or margin of safety). A comparison of the amount of a therapeutic agent that causes the therapeutic effect with the amount that causes toxic effects.

PGI2/TXA2 ratio
A measure that is used to predict the likelihood of thrombus formation, as prostaglandin I2 (PGI2) and thromboxane A2 (TXA2) regulate the interaction between platelets and the vascular wall.

cholesterol or triglyceride levels in hypertensive and diabetic Goto–Kakizaki rats[107], urea-based alkanoic acid sEHIs can transactivate PPARα, which then attenuates vascular smooth muscle cell proliferation[27]. It is also possible that a sEHI with PPARγ agonistic activity could be used for the treatment of cardiometabolic syndrome. This is interesting because PPARγ agonists have the unwanted effect of causing fluid retention that could be detrimental to patients with heart failure and other cardiovascular diseases[129]. However, sEHIs and EETs are natriuretic — that is, they increase Na^+ and water excretion, and could lessen the fluid-retaining state during PPARγ agonist treatment[20,65,79,102]. Overall, there is great potential for sEHIs as antihypertensive treatment that could be used in combination with other medications for patients with poor cardiovascular health, and they may be particularly valuable in patients with co-morbidities.

In addition, sEHIs could have broad neuroprotective actions. There is now mounting evidence that sEHIs provide protection from brain damage following cerebral ischaemia by means that are independent of vascular actions, possibly owing to a sEHI-induced increase in the neuronal expression of pro-survival, anti-apoptotic genes[115]. This is further supported by data showing that ischaemic preconditioning in the brain involves a hypoxia inducible factor-α-mediated increase in expression of the CYP2C11 epoxygenase enzyme in astrocytes[130].

Recent studies also indicate that 14,15-EET activates opioid receptors in the ventrolateral periaqueductal grey area of the brain to produce antinociception[131]. Interestingly, topical application of sEHIs can reduce inflammation-induced pain, which shows the promise of sEHIs as analgesics[66,132]. By contrast, the potential of sEHIs to treat neurological disorders such as Alzheimer's disease or multiple sclerosis has yet to be explored.

Potential for unwanted effects. The potential for unwanted effects must also be considered when developing sEHIs for the treatment of cardiovascular diseases. sEHIs can promote angiogenesis, which could accelerate tumorigenesis in patients with some types of cancer[48,115]. EETs are potent angiogenic lipids that promote vascularization of tumours *in vivo*[48,133,134]. Epoxygenase metabolites have been shown to be a component of the vascular endothelial growth factor-induced, angiogenic endothelial signalling pathways that involve extracellular signal-regulated kinase 1 (ERK1), ERK2, AKT and signal transducer and activator of transcription 3 (REFS 134,135). Although angiogenesis is a potential unwanted effect of sEHI treatment, there are cardiovascular diseases in which angiogenesis would be beneficial. Additionally, these findings have led to the postulate that enhancing sEH activity or inhibiting EET production and/or actions could be an approach for treating various cancers.

Another concern is that sEHIs exacerbate hypoxic pulmonary vasoconstriction and hypoxia-induced pulmonary vascular remodelling[35,36]. Chronic hypoxia elicits pulmonary hypertension and vascular remodelling that is associated with increased EET generation, and epoxygenase inhibition reduces the hypoxic pulmonary vasoconstriction[35,36]. *Ephx2*$^{-/-}$ mice also show an increased pulmonary vasoconstriction in response to hypoxia[35]. The concern of pulmonary hypertension may be limited to that induced by hypoxia as, in monocrotaline-induced pulmonary hypertension, sEHI reduced vascular remodelling and the development of pulmonary hypertension[135]. Another possible concern related to the lungs is that EETs can increase endothelial cell permeability that could result in an unwanted increase in alveolar fluid volume[136,137]. Conversely, 14,15-EET combats TNF-induced hyperreactivity in human airway smooth muscle cells[138]. These findings suggest that sEHIs have the potential unwanted effect of pulmonary vasoconstriction but could be beneficial in treating bronchial inflammation.

There are also potential unwanted cardiovascular effects that could limit the therapeutic utility of sEHIs. Although sEHIs can improve cardiac function following ischaemia, *Ephx2* deletion or sEHIs delayed blood pressure recovery and resulted in higher mortality after cardiopulmonary resuscitation in mice[123]. The effect of sEHI on blood clotting is also complex. Platelet aggregation could be slowed or inhibited, resulting in enhanced bleeding and haemorrhaging in patients taking sEHIs[59,61,139]. However, PGI2/TXA2 ratios and other data provide evidence that sEHIs would speed clotting in animals treated with aspirin but delay clotting in animals treated with rofecoxib (and potentially other COX2 inhibitors)[64,80]. This observation is in agreement with the apparent tendency of EETs to oppose changes in various biological parameters away from the steady state.

Epoxyeicosanoids as a therapeutic target

The fact that sEHIs and EETs are angiogenic and have the potential to increase tumour growth means that inhibiting epoxygenase enzymes or EETs could be a treatment for tumour growth. Interestingly, the CYP2J2 epoxygenase enzyme has been found to be upregulated in many tumours[140]. A recent study exploring the possibility that selective inhibition of the CYP2J2 epoxygenase enzyme would supress tumour growth showed that selective CYP2J2 inhibitors that decreased EET production had marked antitumour properties in *in vitro* and *in vivo* settings, including various human cancer cells[141]. It is also possible that EET antagonists such as 14,15-EEZE, or even the sEH protein, could be used as cancer therapeutics.

Epoxyeicosanoids and EET analogues are also being investigated as potential therapeutic agents for cardiovascular diseases. Increasing EET levels or overexpressing epoxygenase enzymes are cardioprotective[21,65,104,142], which was first shown when addition of 11,12-EET to transplant preservation solutions resulted in improved coronary artery endothelial function[143]. Sulphonamide analogues of EET were developed 15 years ago and other EET analogues and antagonists have been designed and used in *in vitro* perfused vascular and organ tissue experimental studies[31,58,144–146]. Determination of the structure–activity relationships of these EET analogues and antagonists has allowed the requirements for the biological actions of EETs to be determined[58,145,146]. Ultimately, the identification of binding sites or receptors

Cardiometabolic syndrome
A disease state defined as the clustering of visceral obesity with cardiovascular risk factors.

Antinociception
A reduction in sensitivity to painful stimuli.

Pulmonary hypertension
A disease that is characterized by increased pressure in the pulmonary artery.

for EETs could provide new targets for the treatment of cardiovascular diseases. Recent preliminary evidence (from a patent application) suggests that EET analogues can be effectively designed for chronic administration to SHRs, and have antihypertensive actions[147]. A combinational drug that has EET mimetic actions and sEHI activity is another possible approach. Since the finding that certain sEHIs can vasodilate mesenteric resistance arteries, there has been progress in attempts to design EET analogues that can also inhibit the sEH enzyme[148]. EET analogues might also be useful for treating acute myocardial infarction and improving the effectiveness of drug-eluting stents.

Conclusion

Rapid progress has been made in evaluating sEHIs as a therapy for cardiovascular diseases since the first description of their antihypertensive actions in 2000. Future research will be needed to explore other non-cardiovascular diseases that could potentially be treated with sEHIs. There is strong evidence that inflammatory diseases, neurological diseases such as Alzheimer's disease and diseases associated with pain may benefit from sEHI treatment. Therefore, the sEHIs have great potential in the treatment of cardiovascular diseases, and other potential therapeutic applications seem to be on the horizon.

1. Fitzgerald, G. A. Coxibs and cardiovascular disease. *N. Engl. J. Med.* **351**, 1709–1711 (2004).
2. Grosser, T., Fries, S. & FitzGerald, G. A. Biological basis for the cardiovascular consequences of COX-2 inhibition: therapeutic challenges and opportunities. *J. Clin. Invest.* **116**, 4–15 (2006).
3. Puri, A., McGoon, M. D. & Kushwaha, S. S. Pulmonary arterial hypertension: current therapeutic strategies. *Nature Clin. Pract. Cardiovasc. Med.* **4**, 319–329 (2007).
4. Steiropoulos, P., Trakada, G. & Bouros, D. Current pharmacological treatment of pulmonary arterial hypertension. *Curr. Clin. Pharmacol.* **3**, 11–19 (2008).
5. Braden, G. L., O'Shea, M. H., Mulhern, J. G. & Germain, M. J. Acute renal failure and hyperkalaemia associated with cyclooxygenase-2 inhibitors. *Nephrol. Dial. Transplant.* **19**, 1149–1153 (2004).
6. Fries, S. & Grosser, T. The cardiovascular pharmacology of COX-2 inhibition. *Hematology Am. Soc. Hematol. Educ. Program* **2005**, 445–451 (2005).
7. Capra, V. *et al.* Cysteinyl-leukotrienes and their receptors in asthma and other inflammatory diseases: critical update and emerging trends. *Med. Res. Rev.* **27**, 469–527 (2007).
8. Ribeiro, J. D., Toro, A. A. & Baracat, E. C. Anti-leukotrienes in the treatment of asthma and allergic rhinitis. *J. Pediatr. (Rio J.)* **82**, S213–S221 (2006).
9. Capdevila, J., Marnett, L. J., Chacos, N., Prough, R. A. & Estabrook, R. W. Cytochrome P-450-dependent oxygenation of arachidonic acid to hydroxy-icosatetraenoic acids. *Proc. Natl Acad. Sci. USA* **79**, 767–770 (1982).
10. Chacos, N., Falck, J. R., Wixtrom, C. & Capdevila, J. Novel epoxides formed during the liver cytochrome P-450 oxidation of arachidonic acid. *Biochem. Biophys. Res. Commun.* **104**, 916–922 (1982).
11. Oliw, E. H., Lawson, J. A., Brash, A. R. & Oates, J. A. Arachidonic acid metabolism in rabbit renal cortex. Formation of two novel dihydroxyeicosatrienoic acids. *J. Biol. Chem.* **256**, 9924–9931 (1981).
12. Ishizuka, T. *et al.* 20-Hydroxyeicosatetraenoic acid stimulates nuclear factor-κB activation and the production of inflammatory cytokines in human endothelial cells. *J. Pharmacol. Exp. Ther.* **324**, 103–110 (2008).
13. Roman, R. J. P-450 metabolites of arachidonic acid in the control of cardiovascular function. *Physiol. Rev.* **82**, 131–185 (2002).
14. Sarkis, A., Lopez, B. & Roman, R. J. Role of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids in hypertension. *Curr. Opin. Nephrol. Hypertens.* **13**, 205–214 (2004).
15. Renic, M. *et al.* Effect of 20-HETE inhibition on infarct volume and cerebral blood flow after transient middle cerebral artery occlusion. *J. Cereb. Blood Flow Metab.* **29**, 629–639 (2009).
16. Miyata, N. *et al.* Beneficial effects of a new 20-hydroxyeicosatetraenoic acid synthesis inhibitor, TS-011 [N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide], on hemorrhagic and ischemic stroke. *J. Pharmacol. Exp. Ther.* **314**, 77–85 (2005).
17. Campbell, W. B., Gebremedhin, D., Pratt, P. F. & Harder, D. R. Identification of epoxyeicosatrienoic acids as endothelium-derived hyperpolarizing factors. *Circ. Res.* **78**, 415–423 (1996).
This was the first report to show that epoxyeicosatrienoic acids are endothelium-derived hyperpolarizing factors and placed them as important regulators of vascular function.
18. Fleming, I. DiscrEET regulators of homeostasis: epoxyeicosatrienoic acids, cytochrome P450 epoxygenases and vascular inflammation. *Trends Pharmacol. Sci.* **28**, 448–452 (2007).
19. Gross, G. J. *et al.* Effects of the selective EET antagonist, 14,15-EEZE, on cardioprotection produced by exogenous or endogenous EETs in the canine heart. *Am. J. Physiol. Heart Circ. Physiol.* **294**, H2838–H2844 (2008).
20. Imig, J. D. Epoxide hydrolase and epoxygenase metabolites as therapeutic targets for renal diseases. *Am. J. Physiol. Renal Physiol.* **289**, F496–F503 (2005).
21. Spector, A. A., Fang, X., Snyder, G. D. & Weintraub, N. L. Epoxyeicosatrienoic acids (EETs): metabolism and biochemical function. *Prog. Lipid Res.* **43**, 55–90 (2004).
22. Jacobson, H. R. *et al.* in *Prostaglandins and Membrane Ion Transport* (eds Braquet, P. Garay, R. P., Frohlich, J. C. & Nicosia, S) 311–318 (Raven Press, New York, 1985).
23. Proctor, K. G., Falck, J. R. & Capdevila, J. Intestinal vasodilation by epoxyeicosatrienoic acids: arachidonic acid metabolites produced by a cytochrome P450 monooxygenase. *Circ. Res.* **60**, 50–59 (1987).
24. Spector, A. A. Arachidonic acid cytochrome P450 epoxygenase pathway. *J. Lipid Res.* **50**, S52–S56 (2009).
25. Yang, W. *et al.* Characterization of 14,15-epoxyeicosatrienoyl-sulfonamides as 14,15-epoxyeicosatrienoic acid agonists: use for studies of metabolism and ligand binding. *J. Pharmacol. Exp. Ther.* **321**, 1023–1031 (2007).
This study describes the development of EET agonists that could be used for finding EET receptors.
26. Widstrom, R. L., Norris, A. W., Van Der Veer, J. & Spector, A. A. Fatty acid-binding proteins inhibit hydration of epoxyeicosatrienoic acids by soluble epoxide hydrolase. *Biochemistry* **42**, 11762–11767 (2003).
27. Liu, Y. *et al.* The antiinflammatory effect of laminar flow: the role of PPARγ, epoxyeicosatrienoic acids, and soluble epoxide hydrolase. *Proc. Natl Acad. Sci. USA* **102**, 16747–16752 (2005).
28. Michaelis, U. R. & Fleming, I. From endothelium-derived hyperpolarizing factor (EDHF) to angiogenesis: epoxyeicosatrienoic acids (EETs) and cell signaling. *Pharmacol. Ther.* **111**, 584–595 (2006).
29. Spector, A. A. & Norris, A. W. Action of epoxyeicosatrienoic acids on cellular function. *Am. J. Physiol. Cell Physiol.* **292**, C996–C1012 (2007).
30. Fleming, I. Epoxyeicosatrienoic acids, cell signaling and angiogenesis. *Prostaglandins Other Lipid Mediat.* **82**, 60–67 (2007).
31. Breyer, R. M., Bagdassarian, C. K., Myers, S. A. & Breyer, M. D. Prostanoid receptors: subtypes and signaling. *Annu. Rev. Pharmacol. Toxicol.* **41**, 661–690 (2001).
32. Hao, C. M. & Breyer, M. D. Physiological regulation of prostaglandins in the kidney. *Annu. Rev. Physiol.* **70**, 357–377 (2008).
33. Gebremedhin, D. *et al.* Mechanism of action of cerebral epoxyeicosatrienoic acids on cerebral arterial smooth muscle. *Am. J. Physiol.* **263**, H519–H525 (1992).
34. Imig, J. D., Navar, L. G., Roman, R. J., Reddy, K. K. & Falck, J. R. Actions of epoxygenase metabolites on the preglomerular vasculature. *J. Am. Soc. Nephrol.* **7**, 2364–2370 (1996).
35. Keseru, B. *et al.* Epoxyeicosatrienoic acids and the soluble epoxide hydrolase are determinants of pulmonary artery pressure and the acute hypoxic pulmonary vasoconstrictor response. *Faseb J.* **22**, 4306–4315 (2008).
36. Pokreisz, P. *et al.* Cytochrome P450 epoxygenase gene function in hypoxic pulmonary vasoconstriction and pulmonary vascular remodeling. *Hypertension* **47**, 762–770 (2006).
37. Archer, S. L. *et al.* Endothelium-derived hyperpolarizing factor in human internal mammary artery is 11,12-epoxyeicosatrienoic acid and causes relaxation by activating smooth muscle BK_{Ca} channels. *Circulation* **107**, 769–776 (2003).
38. Fisslthaler, B. *et al.* Cytochrome P450 2C is an EDHF synthase in coronary arteries. *Nature* **401**, 493–497 (1999).
39. Li, P. L., Zhang, D. X., Ge, Z. D. & Campbell, W. B. Role of ADP-ribose in 11,12-EET-induced activation of K_{Ca} channels in coronary arterial smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* **282**, H1229–H1236 (2002).
40. Imig, J. D., Inscho, E. W., Deichmann, P. C., Reddy, K. M. & Falck, J. R. Afferent arteriolar vasodilation to the sulfonimide analog of 11,12-epoxyeicosatrienoic acid involves protein kinase A. *Hypertension* **33**, 408–413 (1999).
41. Li, P. L., Chen, C. L., Bortell, R. & Campbell, W. B. 11,12-Epoxyeicosatrienoic acid stimulates endogenous mono-ADP-ribosylation in bovine coronary arterial smooth muscle. *Circ. Res.* **85**, 349–356 (1999).
42. Node, K. *et al.* Activation of Gαs mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. *J. Biol. Chem.* **276**, 15983–15989 (2001).
43. Larsen, B. T. *et al.* Epoxyeicosatrienoic and dihydroxyeicosatrienoic acids dilate human coronary arterioles via BK_{Ca} channels: implications for soluble epoxide hydrolase inhibition. *Am. J. Physiol. Heart Circ. Physiol.* **290**, H491–H499 (2006).
44. Medhora, M. *et al.* Emerging mechanisms for growth and protection of the vasculature by cytochrome P450-derived products of arachidonic acid and other eicosanoids. *Prostaglandins Other Lipid Mediat.* **82**, 19–29 (2007).
45. Medhora, M. *et al.* Epoxygenase-driven angiogenesis in human lung microvascular endothelial cells. *Am. J. Physiol. Heart Circ. Physiol.* **284**, H215–H224 (2003).
46. Potente, M., Fisslthaler, B., Busse, R. & Fleming, I. 11,12-Epoxyeicosatrienoic acid-induced inhibition of FOXO factors promotes endothelial proliferation by down-regulating p27Kip1. *J. Biol. Chem.* **278**, 29619–29625 (2003).
47. Potente, M., Michaelis, U. R., Fisslthaler, B., Busse, R. & Fleming, I. Cytochrome P450 2C9-induced endothelial cell proliferation involves induction of mitogen-activated protein (MAP) kinase phosphatase-1, inhibition of the c-Jun N-terminal kinase, and up-regulation of cyclin D1. *J. Biol. Chem.* **277**, 15671–15676 (2002).
48. Pozzi, A. *et al.* Characterization of 5,6- and 8,9-epoxyeicosatrienoic acids (5,6- and 8,9-EET) as potent *in vivo* angiogenic lipids. *J. Biol. Chem.* **280**, 27138–27146 (2005).

49. Yan, G., Chen, S., You, B. & Sun, J. Activation of sphingosine kinase-1 mediates induction of endothelial cell proliferation and angiogenesis by epoxyeicosatrienoic acids. *Cardiovasc. Res.* **78**, 308–314 (2008).
50. Sun, J. *et al.* Inhibition of vascular smooth muscle cell migration by cytochrome p450 epoxygenase-derived eicosanoids. *Circ. Res.* **90**, 1020–1027 (2002).
51. Davis, B. B. *et al.* Attenuation of vascular smooth muscle cell proliferation by 1-cyclohexyl-3-dodecyl urea is independent of soluble epoxide hydrolase inhibition. *J. Pharmacol. Exp. Ther.* **316**, 815–821 (2006).
52. Davis, B. B. *et al.* Inhibitors of soluble epoxide hydrolase attenuate vascular smooth muscle cell proliferation. *Proc. Natl Acad. Sci. USA* **99**, 2222–2227 (2002).
53. Foley, R. N. & Collins, A. J. End-stage renal disease in the United States: an update from the United States Renal Data System. *J. Am. Soc. Nephrol.* **18**, 2644–2648 (2007).
54. Zoccali, C., Mallamaci, F. & Tripepi, G. Traditional and emerging cardiovascular risk factors in end-stage renal disease. *Kidney Int. Suppl.* **85**, S105–S110 (2003).
55. Elmarakby, A. A. *et al.* Chemokine receptor 2b inhibition provides renal protection in angiotensin II-salt hypertension. *Hypertension* **50**, 1069–1076 (2007).
56. Elmarakby, A. A., Quigley, J. E., Pollock, D. M. & Imig, J. D. Tumor necrosis factor α blockade increases renal Cyp2c23 expression and slows the progression of renal damage in salt-sensitive hypertension. *Hypertension* **47**, 557–562 (2006).
57. Node, K. *et al.* Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. *Science* **285**, 1276–1279 (1999).
This study provided the first description of vascular anti-inflammatory properties of EETs.
58. Falck, J. R. *et al.* 11,12-epoxyeicosatrienoic acid (11,12-EET): structural determinants for inhibition of TNF-α-induced VCAM-1 expression. *Bioorg Med. Chem. Lett.* **13**, 4011–4014 (2003).
59. Fitzpatrick, F. A. *et al.* Inhibition of cyclooxygenase activity and platelet aggregation by epoxy-eicosatrienoic acids. Influence of stereochemistry. *J. Biol. Chem.* **261**, 15334–15338 (1986).
60. Pratt, P. F., Rosolowsky, M. & Campbell, W. B. Effects of epoxyeicosatrienoic acids on polymorphonuclear leukocyte function. *Life Sci.* **70**, 2521–2533 (2002).
61. Heizer, M. L., McKinney, J. S. & Ellis, E. F. 14,15-Epoxyeicosatrienoic acid inhibits platelet aggregation in mouse cerebral arterioles. *Stroke* **22**, 1389–1393 (1991).
62. Kozak, W., Kluger, M. J., Kozak, A., Wachulec, M. & Dokladny, K. Role of cytochrome P-450 in endogenous antipyresis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **279**, R455–R460 (2000).
63. Nakashima, T., Yoshida, Y., Miyata, S. & Kiyohara, T. Hypothalamic 11,12-epoxyeicosatrienoic acid attenuates fever induced by central interleukin-1β in the rat. *Neurosci. Lett.* **310**, 141–144 (2001).
64. Schmelzer, K. R. *et al.* Enhancement of antinociception by coadministration of nonsteroidal anti-inflammatory drugs and soluble epoxide hydrolase inhibitors. *Proc. Natl Acad. Sci. USA* **103**, 13646–13651 (2006).
65. Imig, J. D. Cardiovascular therapeutic aspects of soluble epoxide hydrolase inhibitors. *Cardiovasc. Drug Rev.* **24**, 169–188 (2006).
66. Inceoglu, B. *et al.* Inhibition of soluble epoxide hydrolase reduces LPS-induced thermal hyperalgesia and mechanical allodynia in a rat model of inflammatory pain. *Life Sci.* **79**, 2311–2319 (2006).
67. Zhao, X. *et al.* Soluble epoxide hydrolase inhibition protects the kidney from hypertension-induced damage. *J. Am. Soc. Nephrol.* **15**, 1244–1253 (2004).
This study describes the end-organ-protective properties of sEHIs and provided the first demonstration of a sEHI decreasing renal inflammation associated with hypertension.
68. Smith, K. R. *et al.* Attenuation of tobacco smoke-induced lung inflammation by treatment with a soluble epoxide hydrolase inhibitor. *Proc. Natl Acad. Sci. USA* **102**, 2186–2191 (2005).
69. Fornage, M. *et al.* Polymorphism of the soluble epoxide hydrolase is associated with coronary artery calcification in African-American subjects: The Coronary Artery Risk Development in Young Adults (CARDIA) study. *Circulation* **109**, 335–339 (2004).
This study is the original description of the association of sEH polymorphisms with cardiovascular disease in the human population.
70. Fornage, M. *et al.* The soluble epoxide hydrolase gene harbors sequence variation associated with susceptibility to and protection from incident ischemic stroke. *Hum. Mol. Genet.* **14**, 2829–2837 (2005).
71. Koerner, I. P. *et al.* Polymorphisms in the human soluble epoxide hydrolase gene EPHX2 linked to neuronal survival after ischemic injury. *J. Neurosci.* **27**, 4642–4649 (2007).
72. Lee, C. R. *et al.* Genetic variation in soluble epoxide hydrolase (EPHX2) and risk of coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. *Hum. Mol. Genet.* **15**, 1640–1649 (2006).
73. Srivastava, P. K., Sharma, V. K., Kalonia, D. S. & Grant, D. F. Polymorphisms in human soluble epoxide hydrolase: effects on enzyme activity, enzyme stability, and quaternary structure. *Arch. Biochem. Biophys.* **427**, 164–169 (2004).
74. Wei, Q. *et al.* Sequence variation in the soluble epoxide hydrolase gene and subclinical coronary atherosclerosis: interaction with cigarette smoking. *Atherosclerosis* **190**, 26–34 (2007).
75. Sato, K. *et al.* Soluble epoxide hydrolase variant (Glu287Arg) modifies plasma total cholesterol and triglyceride phenotype in familial hypercholesterolemia: intrafamilial association study in an eight-generation hyperlipidemic kindred. *J. Hum. Genet.* **49**, 29–34 (2004).
76. Dreisbach, A. W. *et al.* The prevalence of CYP2C8, 2C9, 2J2, and soluble epoxide hydrolase polymorphisms in African Americans with hypertension. *Am. J. Hypertens.* **18**, 1276–1281 (2005).
77. Spiecker, M. *et al.* Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. *Circulation* **110**, 2132–2136 (2004).
78. Yu, Z. *et al.* Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* **87**, 992–998 (2000).
This study provided the first experimental evidence that a sEHI can increase epoxide levels and lower blood pressure in an animal model of hypertension.
79. Imig, J. D., Zhao, X., Capdevila, J. H., Morisseau, C. & Hammock, B. D. Soluble epoxide hydrolase inhibition lowers arterial blood pressure in angiotensin II hypertension. *Hypertension* **39**, 690–694 (2002).
80. Imig, J. D. *et al.* An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* **46**, 975–981 (2005).
This study describes the first oral administration of a sEHI and was the first to show the antihypertensive and end-organ-protective effects of sEHIs.
81. Morisseau, C. & Hammock, B. D. Gerry Brooks and epoxide hydrolases: four decades to a pharmaceutical. *Pest Manag. Sci.* **64**, 594–609 (2008).
82. Newman, J. W., Morisseau, C. & Hammock, B. D. Epoxide hydrolases: their roles and interactions with lipid metabolism. *Prog. Lipid Res.* **44**, 1–51 (2005).
83. Oesch, F., Schladt, L., Hartmann, R., Timms, C. & Worner, W. Rat cytosolic epoxide hydrolase. *Adv. Exp. Med. Biol.* **197**, 195–201 (1986).
84. EnayetAllah, A. E. *et al.* Opposite regulation of cholesterol levels by the phosphatase and hydrolase domains of soluble epoxide hydrolase. *J. Biol. Chem.* **283**, 36592–36598 (2008).
85. Morisseau, C. & Hammock, B. D. Epoxide hydrolases: mechanisms, inhibitor designs, and biological roles. *Annu. Rev. Pharmacol. Toxicol.* **45**, 311–333 (2005).
86. Newman, J. W., Morisseau, C., Harris, T. R. & Hammock, B. D. The soluble epoxide hydrolase encoded by EPXH2 is a bifunctional enzyme with novel lipid phosphate phosphatase activity. *Proc. Natl Acad. Sci. USA* **100**, 1558–1563 (2003).
87. Enayetallah, A. E., French, R. A., Thibodeau, M. S. & Grant, D. F. Distribution of soluble epoxide hydrolase and of cytochrome P450 2C8, 2C9, and 2J2 in human tissues. *J. Histochem. Cytochem.* **52**, 447–454 (2004).
88. Yu, Z. *et al.* Vascular localization of soluble epoxide hydrolase in the human kidney. *Am. J. Physiol. Renal Physiol.* **286**, F720–F726 (2004).
89. Harris, T. R. *et al.* Identification of two epoxide hydrolases in *Caenorhabditis elegans* that metabolize mammalian lipid signaling molecules. *Arch. Biochem. Biophys.* **472**, 139–149 (2008).
90. Tran, K. L. Lipid sulfates and sulfonates are allosteric competitive inhibitors of the N-terminal phosphatase activity of the mammalian soluble epoxide hydrolase. *Biochemistry* **44**, 12179–12187 (2005).
91. Harris, T. R., Aronov, P. A. & Hammock, B. D. Soluble epoxide hydrolase homologs in *Strongylocentrotus purpuratus* suggest a gene duplication event and subsequent divergence. *DNA Cell Biol.* **27**, 467–477 (2008).
92. Mullin, C. A. & Hammock, B. D. Chalcone oxides — potent selective inhibitors of cytosolic epoxide hydrolase. *Arch. Biochem. Biophys.* **216**, 423–439 (1982).
93. Morisseau, C. *et al.* Potent urea and carbamate inhibitors of soluble epoxide hydrolases. *Proc. Natl Acad. Sci. USA* **96**, 8849–8854 (1999).
This is the original description of the development of urea compounds as sEHIs.
94. Kim, I. H. *et al.* Optimization of amide-based inhibitors of soluble epoxide hydrolase with improved water solubility. *J. Med. Chem.* **48**, 3621–3629 (2005).
95. Morisseau, C. *et al.* Development of metabolically stable inhibitors of mammalian microsomal epoxide hydrolase. *Chem. Res. Toxicol.* **21**, 951–957 (2008).
96. Xie, Y. *et al.* Discovery of potent non-urea inhibitors of soluble epoxide hydrolase. *Bioorg. Med. Chem. Lett.* **19**, 2354–2359 (2009).
97. Liu, J.-Y. *et al.* Sorafenib has soluble epoxide hydrolase inhibitory activity which contributes to its effect profile *in vivo*. *Mol. Cancer Ther.* **8**, 2193–2203 (2009).
98. Ghosh, S. *et al.* Oral delivery of 1,3-dicyclohexylurea nanosuspension enhances exposure and lowers blood pressure in hypertensive rats. *Basic Clin. Pharmacol. Toxicol.* **102**, 453–458 (2008).
99. Morisseau, C. *et al.* Structural refinement of inhibitors of urea-based soluble epoxide hydrolases. *Biochem. Pharmacol.* **63**, 1599–1608 (2002).
100. Hwang, S. H., Tsai, H. J., Liu, J. Y., Morisseau, C. & Hammock, B. D. Orally bioavailable potent soluble epoxide hydrolase inhibitors. *J. Med. Chem.* **50**, 3825–3840 (2007).
101. Ai, D. *et al.* Soluble epoxide hydrolase plays an essential role in angiotensin II-induced cardiac hypertrophy. *Proc. Natl Acad. Sci. USA* **106**, 564–569 (2009).
102. Jung, O. *et al.* Soluble epoxide hydrolase is a main effector of angiotensin II-induced hypertension. *Hypertension* **45**, 759–765 (2005).
103. Koerner, I. P. *et al.* Soluble epoxide hydrolase: regulation by estrogen and role in the inflammatory response to cerebral ischemia. *Front. Biosci.* **13**, 2833–2841 (2008).
104. Seubert, J. M. *et al.* Role of soluble epoxide hydrolase in postischemic recovery of heart contractile function. *Circ. Res.* **99**, 442–450 (2006).
This study used the combination of genetic and pharmacological manipulation of sEHIs and epoxides and showed cardiac-protective effects from ischaemic events.
105. Ulu, A. *et al.* Soluble epoxide hydrolase inhibitors reduce the development of atherosclerosis in apolipoprotein E-knockout mouse model. *J. Cardiovasc. Pharmacol.* **52**, 314–323 (2008).
106. Marino, J. P. Jr. Soluble epoxide hydrolase, a target with multiple opportunities for cardiovascular drug discovery. *Curr. Top. Med. Chem.* **9**, 452–463 (2009).
107. Olearczyk, J. J. *et al.* Administration of a substituted adamantyl urea inhibitor of soluble epoxide hydrolase protects the kidney from damage in hypertensive Goto-Kakizaki rats. *Clin. Sci. (Lond.)* **116**, 61–70 (2009).
108. Motoki, A. *et al.* Soluble epoxide hydrolase inhibition and gene deletion are protective against myocardial ischemia-reperfusion injury *in vivo*. *Am. J. Physiol. Heart Circ. Physiol.* **295**, H2128–H2134 (2008).
109. Fife, K. L. *et al.* Inhibition of soluble epoxide hydrolase does not protect against endotoxin-mediated hepatic inflammation. *J. Pharmacol. Exp. Ther.* **327**, 707–715 (2008).
110. Katragadda, D. *et al.* Epoxyeicosatrienoic acids limit damage to mitochondrial function following stress in cardiac cells. *J. Mol. Cell Cardiol.* **46**, 867–875 (2009).
111. Loch, D., Hoey, A., Morisseau, C., Hammock, B. O. & Brown, L. Prevention of hypertension in DOCA-salt rats by an inhibitor of soluble epoxide hydrolase. *Cell Biochem. Biophys.* **47**, 87–98 (2007).
112. Fleming, I. Vascular cytochrome p450 enzymes: physiology and pathophysiology. *Trends Cardiovasc. Med.* **18**, 20–25 (2008).
113. Fornage, M. *et al.* Polymorphism in soluble epoxide hydrolase and blood pressure in spontaneously hypertensive rats. *Hypertension* **40**, 485–490 (2002).

114. Dorrance, A. M. *et al.* An epoxide hydrolase inhibitor, 12-(3-adamantan-1-yl-ureido)dodecanoic acid (AUDA), reduces ischemic cerebral infarct size in stroke-prone spontaneously hypertensive rats. *J. Cardiovasc. Pharmacol.* **46**, 842–848 (2005).
115. Simpkins, A. N. *et al.* Soluble epoxide hydrolase inhibition is protective against cerebral ischemia via vascular and neural protection. *Am. J. Pathol.* **174**, 2086–2095 (2009).
116. Corenblum, M. J. *et al.* Altered soluble epoxide hydrolase gene expression and function and vascular disease risk in the stroke-prone spontaneously hypertensive rat. *Hypertension* **51**, 567–573 (2008).
117. Sinal, C. J. *et al.* Targeted disruption of soluble epoxide hydrolase reveals a role in blood pressure regulation. *J. Biol. Chem.* **275**, 40504–40510 (2000).
118. Luria, A. *et al.* Compensatory mechanism for homeostatic blood pressure regulation in Ephx2 gene-disrupted mice. *J. Biol. Chem.* **282**, 2891–2898 (2007).
119. Manhiani, M. *et al.* Soluble epoxide hydrolase gene deletion attenuates renal injury and inflammation with DOCA-salt hypertension. *Am. J. Physiol. Renal Physiol.* 24 Jun 2009 (doi:10.1152/ajprenal.00098.2009).
120. Parrish, A. R. *et al.* Attenuation of cisplatin nephrotoxicity by inhibition of soluble epoxide hydrolase. *Cell Biol. Toxicol.* **25**, 217–225 (2008).
121. Xu, D. *et al.* Prevention and reversal of cardiac hypertrophy by soluble epoxide hydrolase inhibitors. *Proc. Natl Acad. Sci. USA* **103**, 18733–18738 (2006).
122. Li, J. *et al.* Soluble epoxide hydrolase inhibitor, AUDA, prevents early salt-sensitive hypertension. *Front. Biosci.* **13**, 3480–3487 (2008).
123. Hutchens, M. P. *et al.* Soluble epoxide hydrolase gene deletion reduces survival after cardiac arrest and cardiopulmonary resuscitation. *Resuscitation* **76**, 89–94 (2008).
124. Zhang, W. *et al.* Soluble epoxide hydrolase gene deletion is protective against experimental cerebral ischemia. *Stroke* **39**, 2073–2078 (2008).
125. Zhang, W. *et al.* Soluble epoxide hydrolase: a novel therapeutic target in stroke. *J. Cereb. Blood Flow Metab.* **27**, 1931–1940 (2007).
126. Ai, D. *et al.* Angiotensin II up-regulates soluble epoxide hydrolase in vascular endothelium *in vitro* and *in vivo*. *Proc. Natl Acad. Sci. USA* **104**, 9018–9023 (2007).
127. Schmelzer, K. R. *et al.* Soluble epoxide hydrolase is a therapeutic target for acute inflammation. *Proc. Natl Acad. Sci. USA* **102**, 9772–9777 (2005).
128. Fang, X. *et al.* Activation of peroxisome proliferator-activated receptor alpha by substituted urea-derived soluble epoxide hydrolase inhibitors. *J. Pharmacol. Exp. Ther.* **314**, 260–270 (2005).
129. Buckingham, R. E. & Hanna, A. Thiazolidinedione insulin sensitizers and the heart: a tale of two organs? *Diabetes Obes. Metab.* **10**, 312–328 (2008).
130. Liu, M. & Alkayed, N. J. Hypoxic preconditioning and tolerance via hypoxia inducible factor (HIF) 1α-linked induction of P450 2C11 epoxygenase in astrocytes. *J. Cereb. Blood Flow Metab.* **25**, 939–948 (2005).
131. Terashvili, M. *et al.* Antinociception produced by 14,15-epoxyeicosatrienoic acid is mediated by the activation of β-endorphin and met-enkephalin in the rat ventrolateral periaqueductal gray. *J. Pharmacol. Exp. Ther.* **326**, 614–622 (2008).
132. Iceoglu, B. *et al.* Soluble epoxide hydrolase and epoxyeicosatrienoic acids modulate two distinct analgesic pathways. *Proc. Natl Acad. Sci. USA* **105**, 18901–18906 (2008).
133. Yang, S., Wei, S., Pozzi, A. & Capdevila, J. H. The arachidonic acid epoxygenase is a component of the signaling mechanisms responsible for VEGF-stimulated angiogenesis. *Arch. Biochem. Biophys.* 21 May 2009 (doi:10.1016/j.abb.2009.05.006).
134. Cheranov, S. Y. *et al.* An essential role for SRC-activated STAT-3 in 14,15-EET-induced VEGF expression and angiogenesis. *Blood* **111**, 5581–5591 (2008).
135. Revermann, M. *et al.* Inhibition of the soluble epoxide hydrolase attenuates monocrotaline-induced pulmonary hypertension in rats. *J. Hypertens.* **27**, 322–331 (2009).
136. Alvarez, D. F., Gjerde, E. A. & Townsley, M. I. Role of EETs in regulation of endothelial permeability in rat lung. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **286**, L445–L451 (2004).
137. Jian, M. Y., King, J. A., Al-Mehdi, A. B., Liedtke, W. & Townsley, M. I. High vascular pressure-induced lung injury requires P450 epoxygenase-dependent activation of TRPV4. *Am. J. Respir. Cell. Mol. Biol.* **38**, 386–392 (2008).
138. Morin, C., Sirois, M., Echave, V., Gomes, M. M. & Rousseau, E. EET displays anti-inflammatory effects in TNF-α stimulated human bronchi: putative role of CPI-17. *Am. J. Respir. Cell. Mol. Biol.* **38**, 192–201 (2008).
139. Krotz, F. *et al.* Membrane-potential-dependent inhibition of platelet adhesion to endothelial cells by epoxyeicosatrienoic acids. *Arterioscler. Thromb. Vasc. Biol.* **24**, 595–600 (2004).
140. Jiang, J. G. *et al.* Cytochrome P450 2J2 promotes the neoplastic phenotype of carcinoma cells and is up-regulated in human tumors. *Cancer Res.* **65**, 4707–4715 (2005).
141. Chen, C. *et al.* Selective inhibitors of CYP2J2 related to terfenadine exhibit strong activity against human cancers *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.* **329**, 908–918 (2009).
142. Seubert, J. *et al.* Enhanced postischemic functional recovery in CYP2J2 transgenic hearts involves mitochondrial ATP-sensitive K^+ channels and p42/p44 MAPK pathway. *Circ. Res.* **95**, 506–514 (2004).
143. Yang, Q., Zhang, R. Z., Yim, A. P. & He, G. W. Effect of 11,12-epoxyeicosatrienoic acid as an additive to St. Thomas' cardioplegia and University of Wisconsin solutions on endothelium-derived hyperpolarizing factor-mediated function in coronary microarteries: influence of temperature and time. *Ann. Thorac. Surg.* **76**, 1623–1630 (2003).
144. Falck, J. R. *et al.* Comparison of vasodilatory properties of 14,15-EET analogs: structural requirements for dilation. *Am. J. Physiol. Heart Circ. Physiol.* **284**, H337–H349 (2003).
145. Gauthier, K. M., Falck, J. R., Reddy, L. M. & Campbell, W. B. 14,15-EET analogs: characterization of structural requirements for agonist and antagonist activity in bovine coronary arteries. *Pharmacol. Res.* **49**, 515–524 (2004).
146. Imig, J. D., Dimitropoulou, C., Reddy, D. S., White, R. E. & Falck, J. R. Afferent arteriolar dilation to 11,12-EET analogs involves PP2A activity and $Ca2^+$-activated K^+ channels. *Microcirculation* **15**, 137–150 (2008).
147. Imig, J. D. & Falck, J. R. Compositions and methods for the treatment of renal and cardiovascular disease. US Patent 2008146663 (2008).
148. Olearczyk, J. J. *et al.* Substituted adamantyl-urea inhibitors of the soluble epoxide hydrolase dilate mesenteric resistance vessels. *J. Pharmacol. Exp. Ther.* **318**, 1307–1314 (2006).

Competing interests statement
The authors declare competing financial interests: see web version for details.

DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
EPHX2
UniProtKB: http://www.uniprot.org
ALOX5 | AKT | COX2 | PI3K | TNF | VCAM1

FURTHER INFORMATION

John Imig's homepage:
http://www.mcw.edu/display/docid24736.htm
Bruce Hammock's homepage:
http://www.biopestlab.ucdavis.edu/
Arete Therapeutics initiates Phase IIa clinical trial for AR9281, a novel sEH inhibitor to treat type 2 diabetes:
http://www.aretetherapeutics.com/news/2009/021009.html

ALL LINKS ARE ACTIVE IN THE ONLINE PDF