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Review Article

The roles of cytochromes P450 in vascular biology and cardiovascular homeostasis

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Abstract: Cardiovascular disease and atherosclerosis are human health crises that remain the leading cause of death worldwide. The cytochromes P450 (CYPs) are key metabolizing enzymes of both xenobiotics and endobiotics. Many CYP enzymes have been identified in the heart, kidneys, endothelium, and smooth muscle of blood vessels. Furthermore, mounting evidence points to the role of endogenous CYP-dependent metabolites, such as epoxyeicosatrienoic acids (EETs), 20-hydroxyeicosatetraenoic acid (20-HETE), thromboxane A_2 (TxA_2) and prostacyclin (PGI_2), in the maintenance of vascular physiology and cardiovascular homeostasis. The link between CYP genetic polymorphism and its pathological impact on cardiovascular disorders, such as hypertension and myocardial infarction, has been established in recent years. Therefore, there are numerous studies indicating the involvement of CYP in atherosclerotic and cardiovascular diseases. Currently, concentrating on treatment modalities that target the CYP pathways represents an attractive therapeutic strategy for the researchers in the field. While data are promising, further clinical investigation is necessary to understand fully the functional roles of the CYP enzymes in the regulation of vasculature and cardiovascular injuries in humans and to validate the safety of these potential therapeutic agents for use in patients.

Keywords: Arachidonic acid, cardiovascular homeostasis, cytochrome P450, eicosanoids, novel therapeutic agents, polymorphisms, prostanoids

Introduction

The cytochrome P450 (CYP) superfamily is one group of important Phase I metabolizing enzymes that oxidize a number of endogenous compounds and xenobiotics, including more than 90% of clinically used drugs [1]. Endogenous compounds metabolized by CYPs include fatty acids, steroids, fat-soluble vitamins, bile acids, and many other lipophilic compounds. While the roles of CYP enzymes in the metabolism of xenobiotics have been well established, their role in extra-hepatic tissues including heart, kidneys and brain in endogenous tissue function has been started to be appreciated in recent decades [2]. Evidences suggest that metabolism mediated by CYP enzymes plays an important role in the homeostasis of many body organ systems including blood, cardiovascular, renal, and nervous systems.

This review is intended to provide an overview of the role of CYP enzymes in organ homeostasis with particular focus on vasculature and cardiovascular systems. The review starts with a discussion on the CYP epoxygenase, ω-hydroxylase and prostanoid synthase pathways followed by a description of the role of CYP enzymes (and their genetic polymorphisms) in vascular biology and pathophysiology. Finally this review highlights therapeutic strategies aimed at preventing or reducing the mortality resulting from vascular and cardiovascular diseases.

CYP eicosanoid metabolism pathways

Discussing the role of CYP in vascular biology and cardiovascular homeostasis would be incomplete without mentioning their role in eicosanoid metabolism. It is well established that CYP-derived eicosanoid metabolites play

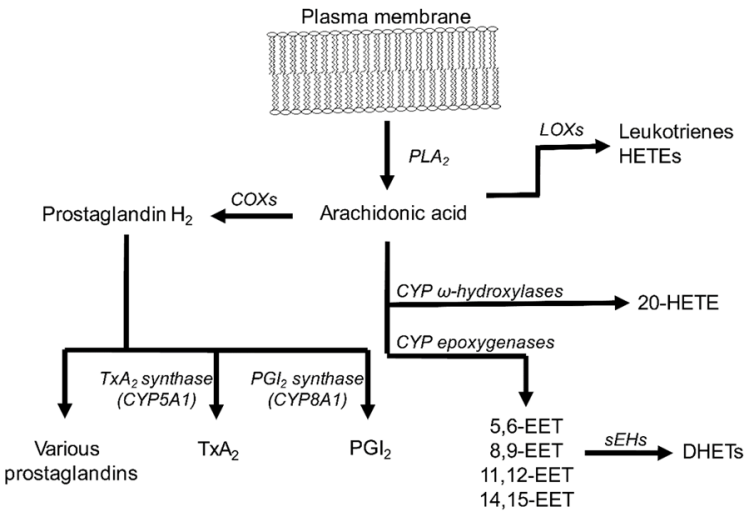

Figure 1. The eicosanoid biosynthetic pathways. Arachidonic acid (AA) is a polyunsaturated omega-6 fatty acid which is released from the membrane phospholipids by the activity of phospholipases such as cytosolic phospholipase A_2 (PLA_2). Free AA can be metabolized to eicosanoids through three major pathways: the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway, and the cytochrome P450 (CYP) epoxygenase and ω-hydroxylase pathways. In COX pathway, thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) are further generated by the TxA_2 synthase (CYP5A1) and PGI_2 synthase (CYP8A1) respectively. In the CYP pathways, AA is converted to epoxyeicosatrienoic acids (EETs) and 20-hydroxyeicosatetraenoicacid (20-HETE) by CYP epoxygenases and CYP ω-hydroxylases, respectively. All EETs are then further metabolized by soluble epoxide hydrolase (sEH) forming the less active dihydroxyeicosatrienoic acids (DHETs).

important blood and cardiovascular functions; two prostanoids, thromboxane A_2 (TxA_2) and prostacyclin (PGI_2), are instrumental in atherogenesis, while epoxyeicosatrienoic acids (EETs) and hydroxyeicosatetraenoic acids (HETEs) play a pivotal role in the maintenance of cardiovascular biological function.

Arachidonic acid (AA), the source of the above mentioned metabolites, is a 20-carbon, omega-6 polyunsaturated fatty acid (PUFA) found in the cell membrane phospholipids and is released when stimulated by the cytosolic enzyme phospholipase A_2 [3]. As illustrated in **Figure 1**, free AA can be metabolized to eicosanoids through the following three major pathways: the cyclooxygenase (COX) pathway, which generates prostanoids; lipoxygenase (LOX) pathway, which generates leukotrienes and HETEs; and lastly, CYP pathways, which involve CYP epoxygenase and CYP ω-hydroxylase enzymes [4, 5]. The synthesis of prostanoids involves two CYP isoforms, CYP5A1 and CYP8A1. More popularly known as thromboxane A_2 synthase [6], CYP5A1 is involved in platelet aggregation, whereas CYP8A1, conventionally known as prostacyclin (PGI_2) synthase [7], participates in platelet disaggregation. Therefore, CYP5A1 and CYP8A1 provide a Yin-Yang system in regulating the blood coagulation process and atherogenesis. CYP epoxygenase enzymes, on the other hand, are involved in generating bioactive EETs [8]. CYP2J and CYP2C epoxygenases are important sources of all four EET regioisomers (5,6-, 8,9-, 11,12-, and 14,15-EETs) with each regioisomer being composed of two different stereoisomers. CYP2J2, CYP2C8, and CYP2C9 are constitutively and abundantly expressed in the human heart tissue [9]. All EETs are then further biotransformed by soluble epoxide hydrolase (sEH) [4] to form the less active dihydroxyeicosatrienoic acids (DHETs). Most biological effects of EETs are attenuated by this process, making sEH a target for increasing and prolonging the actions of EETs [10]. CYP ω-hydroxylases catalyze ω-terminal hydroxylation by forming C16-C20 alcohols of AA (16-, 17-, 18-, 19-, and 20-HETEs). Of particular importance are CYP4A and CYP4F enzymes that mainly catalyze the formation of 20-HETE, a vasoactive and natriuretic eicosanoid important for vascular function [11].

In addition to AA-derived products, other members of the omega-6 PUFA family (most notably linoleic acid) and of the omega-3 PUFA family, such as docosahexaenoic acid and eicosapentaenoic acid, play a role in many other physiological processes [12]. These roles are beyond the scope of this review and are not discussed further.

The role of CYPs in cardiovascular and vascular diseases

Atherosclerosis

Atherosclerosis is the primary cause of coronary artery disease, as well as cerebral and peripheral vascular diseases [13]. Atherosclerotic pathogenesis involves chronic and pro-

gressive vascular inflammation accompanied by complex pathological mechanisms including endothelial dysfunction, dyslipidemia and platelet hyperactivity [14]. The bioactive eicosanoids play an important role in modulating atherosclerotic processes, and among these lipids, TxA_2 and PGI_2 and their receptors clearly play a functional role in atherogenesis [15].

From biochemical perspective, TxA_2 and PGI_2 are generated in two steps from AA *via* the COX pathway and subsequent action of thromboxane A_2 and PGI_2 synthase (or CYP5A1 and CYP8A1), respectively (see **Figure 1**). TxA_2 and PGI_2 act through binding to specific G protein-coupled prostanoid receptors called TP and IP receptors, respectively. Both are membrane bound, seven transmembrane receptors distributed widely in the vasculature and cardiovascular systems [16]. The biological effects mediated by these receptors are well established; as a strong activator of platelets, a vasoconstrictor and smooth muscle cell mitogen, TxA_2 is mainly produced in blood platelets but can also be synthesized by monocytes and vascular smooth muscle cells [17]. PGI_2, which is produced mainly by vascular endothelial cells, acts as a biological opposite to TxA_2 and has potent platelet-inhibitory and vasodilating properties [18]. Accumulating data demonstrate an increased formation of these two bioactive lipids and the alteration of their enzymatic machinery and receptors in atherosclerotic vascular events. As an example, there is a significant increase in TP receptor expression in the aorta and epicardial coronary arteries from ischemic atherosclerotic vessels [19]. TP receptors, when activated, trigger a series of defensive endothelial and inflammatory responses, including enhanced expression of adhesion and chemotactic molecules (e.g., P-selectin, intracellular adhesion molecule [ICAM-1], vascular cell adhesion molecule [VCAM]), vascular smooth muscle contraction, chemotaxis of monocytes and activation to macrophages, release of inflammatory mediators, matrix metalloproteinases (MMPs), and various tissue factors that collectively generate the necrotic lipid core at sub-endothelial space, eventually resulting in advanced atherosclerotic lesions [15]. Besides endothelial dysfunction and inflammation, platelet activation significantly contributes to plaque formation and atherosclerosis progression [20]. Platelets increase the expression of adhesion molecules, further providing the reactive surface for lymphocytes recruitment. They also serve as a source of growth factors (i.e., platelet-derived growth factor), and proinflammatory cytokines (e.g., CD40 ligand and interleukins) that perpetuate the inflammatory milieu within the plaque.

Due to biological opposite nature of TxA_2 and PGI_2, the balance between these two mediators is important in both healthy and diseased vasculature. Different from TxA_2, PGI_2 has been shown to modulate *in vivo* platelet-vascular interactions in an opposie manner, thus its ability to specifically limit the response to TxA_2 [15]. In view of this, factors that modulate their biological activities would cause alteration in vascular function. A clear example is the Ghosalhaemato-diaphyseal syndrome, a rare autosomal recessive trait, which is due to mutations in the *CYP5A1* gene [21]. The dysfunction in this gene impacts negatively on platelet aggregation and atherogenesis. Besides demonstrating deficit in platelet aggregation, patients with this syndrome also exhibit altered bone density, progressive sclerosing dysplasia in the midsection of long bones and refractory anaemia, all of which are associated with blood and vascular dysfunction. Another example is the association of essential hypertension and cerebral infarction with mutations in *CYP8A1* gene [22, 23]. Japanese families with mutation and polymorphism of the *CYP8A1* gene were found to display a history of hypertension and cerebral infarction in these studies, suggesting that abnormal CYP8A1 activity may lead to altered vasodilation and platelet aggregation.

Similar to TxA_2 and PGI_2, other eicosanoid metabolites including EETs and 20-HETE are also instrumental in the process of atherogenesis and pathogenesis of cardiovascular diseases. Their role is further elaborated in the next few sections below.

Ischemic heart disease

The role of different CYP isoforms in cardiovascular homeostasis and pathophysiology of ischemic heart disease (IHD) is now well established. CYP epoxygenases, in particular, isoforms in the CYP2C and 2J subfamilies are mainly involved in the conversion of AA into EETs in cardiovascular tissues. EETs exert

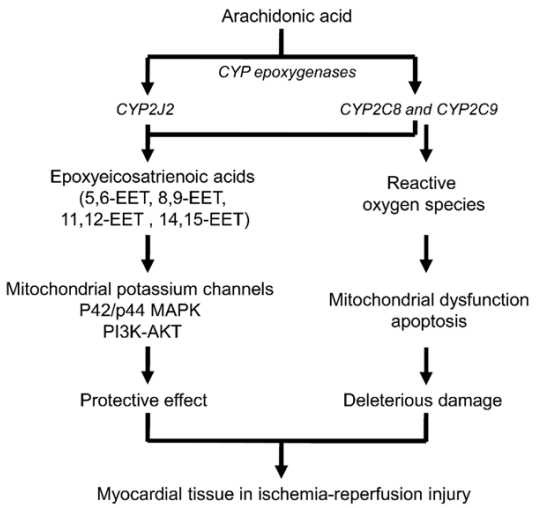

Figure 2. Role of CYP epoxygenases in ischemia-perfusion injury of human myocardial tissue.

numerous cardioprotective effects, including vasodilatory, antiinflammatory, antiapoptotic, antithrombotic and natriuretic actions [24].

The impaired endothelial function and abnormal coronary blood flow regulation in IHD eventually lead to the onset and progression of atherosclerosis and thrombosis [25]. Several lines of evidence have demonstrated that CYP-derived EETs serve as endothelial-derived hyperpolarizing factor (EDHF) in the coronary circulation [26]. EDHF induces hyperpolarization in vascular smooth muscle cells by activating the large-conductance calcium-activated potassium (BK) channels as well as Na^+-K^+-ATPase, resulting in vasodilation. Plasma concentrations of EETs have been shown to be higher in patients with IHD than in healthy controls [27]. Increased concentrations of EETs may be the result of a compensatory rise of this vasodilator in the presence of advanced atherosclerosis. The key role of CYPs and EDHF is further evidenced from their expression modulation by the dihydropyridine calcium channel blocker nifedipine. This drug has been shown to increase the expression of CYP2C, but not CYP2J or CYP2B, in endothelial layer of coronary arteries. Moreover, it was able to mediate EET synthesis increase and to potentiate hyperpolarization of coronary artery smooth muscle cells mediated through bradykinin and EDHF [28].

The role of 20-HETE is less well established in the case of IHD. No difference in 20-HETE levels was observed in patients with IHD; however, these patients exhibited significantly higher EET levels than healthy controls [13]. This suggests that the CYP-mediated eicosanoid metabolism is deregulated in certain patients with IHD, and demonstrates that the biomarkers of CYP epoxygenase and sEH, but not CYP ω-hydroxylase metabolism are altered in patients with IHD relative to healthy individuals.

EETs also play a key role in ischemia-reperfusion injury (IRI), exacerbation of myocardial damage induced by blood flow restoration and tissue reperfusion after an infarction event [29]. Accumulating data in recent years indicate the importance of EETs in protecting the heart from IRI (see **Figure 2**). The direct administration of EETs to the coronary circulation resulted in a significant decrease of infarct size and myocardial remodeling after IRI in animal models [30]. The attenuation of deleterious electrocardiographic changes (such as QT prolongation and ST elevation) and mitochondrial dysfunction (increased mitochondrial T-tube swelling and fragmentation) were also noted following ischemia-reperfusion of the isolated perfused heart [31, 32]. These protective effects were blocked by the administration of the EET antagonist 14,15-epoxyeicosa-5(Z)-enoic acid. The cardioprotective mechanism of EETs are linked to the activation of multiple pathways including sarcolemmal and mitochondrial potassium channels, p42/p44 mitogen-activated protein kinase (MAPK), and phosphoinositide-3-kinase-protein kinase B (PI3K-AKT) signaling [32, 33], all of which are well characterized mechanisms for cardioprotection.

Recent evidences point to a different role of CYP2 isoforms in IRI of the heart. While enhanced EET level and over-expression of CYP2J2 improved the functional recovery from IRI, CYP2C9 and CYP2C8, have been associated with a poor outcome of ischemia-reperfusion [34]. Both CYP2C isoforms have been shown to generate reactive oxygen species (ROS), which accelerated ischemic damage in tissues (see **Figure 2**). This can be explained by the fact that a CYP epoxygenase is not always just an epoxygenase because superoxide anions (O_2^-), hydrogen peroxide, and hydroxyl radicals can also be generated during the CYP catalysis when electrons for the reduction of the central heme iron are transferred to the

activated bound oxygen molecule [35]. More specifically, the over-expression of human CYP2C9 was found to increase ROS formation in endothelial cells, and this was completely inhibited by the administration of the CYP2C9-selective inhibitor sulfaphenazole [36]. In addition, sulfaphenazole enhanced the endothelium-dependent vasodilator response in patients with coronary artery disease possibly *via* the suppression of ROS formation [37]. In another study, mice with selective increased endothelial expression of CYP2C8 showed decreased functional recovery and increased infarct size after ischemia-reperfusion [38]. The above data indicate that the type of isoform is critical for ROS generation as well as EET production in myocardial tissue. The nature and severity of IRI would thus depend on the fine balance of the expression and activity profiles of the CYP2C and CYP2J isoforms in the vascular and cardiac tissues concerned.

Genetic polymorphisms of CYP enzymes are expected to have a pathological impact on IHD in view of their important role in eicosanoid metabolism. Patients with CYP variants would thus have an altered capacity to generate EETs, influencing their vulnerability to IHD [39]. Several association studies conducted in the past have provided evidence for this vulnerability. *CYP2C9*2* and *CYP2C9*3*, were associated with the risk of acute myocardial infarction in female patients [40]. *CYP2C8*3* was also linked to a high risk of myocardial infarction in both males and females [40]. Furthermore, *CYP2C8*3* was found to increase the incidence of coronary artery disease in smokers [41]. This same study also demonstrated that the *CYP2J2* G-50T polymorphism variant -50T allele was associated with significantly lower risk of coronary heart disease in African Americans. Another study demonstrated that *CYP2J2* polymorphism was an independent risk factor of IHD [42]. However, the positive associations reported above are not supported by more recent studies, which indicated no association for *CYP2C8* and *CYP2C9* [43, 44]. Further studies that include more ethnic groups or additional independent population cohorts are required to clarify these associations. In addition, more careful consideration of the potential role of clinical and demographic confounders suchas phenotypic definition, ethnic admixture and population stratification is warranted in the design of such studies.

Hypertension

There is substantial evidence for the role of enzymes in the EET and HETE pathways, including CYPs and sEHs, in the pathophysiology of hypertension. The most convincing evidence was the report that male animals lacking sEH demonstrated an elevated circulating level of EETs, decreased renal production of DHETs (EET metabolites) and a significantly lower blood pressure [45]. Moreover, significant lowering of blood pressure was noted in various rodent models of hypertension following the administration of a sEH inhibitor [45, 46]. Accumulating data [47, 48] have also demonstrated that transgenic or overexpression of CYP epoxygenase lowers blood pressure in several animal hypertensive models. For example, recombinant adeno-associated virus vector-mediated overexpression of CYP2J2 prevented the development of hypertension in spontaneously hypertensive rats [47]. Furthermore, the *in vivo* delivery of CYP2J3, a homologous gene of CYP2J2 expressed mainly in rats, increased EET level and reduced blood pressure in rats treated with fructose [48]. The vasodilatory effect of EETs is contrasted by 20-HETE, which plays a prohypertensive role. This is evidenced from blood pressure elevation in animal models by infusion of the angiotensin II, a potent vasoconstrictor [49]. It has been established that angiotensin II stimulates 20-HETE synthesis in renal microvessels and decreases EET levels by down regulating epoxygenases and increasing their degradation by increasing expression and activity of sHE [50, 51].

Several mechanisms by which EETs can potentially affect blood pressure have been demonstrated. These include direct effects on the vasculature and regulation of renal sodium excretion by modulating the expression and activity of ion channels such as BK channels, inwardly rectifying K channels, or epithelial sodium channels ($E_{Na}Cs$) [52]. The activation of BK channels has been reported to lead to hyperpolarization and relaxation of vascular smooth muscle cells [53]. Besides effects on the basal blood pressure, EETs can affect blood pressure *via* the modulation of the renin-angiotensin system. For example, sEH inhibitors are very effective in the treatment of hypertension associated with activation of the

renin-angiotensin system [54]. This is due to the fact that angiotensin II is a potent inducer of sEH expression in the rat vasculature [55] as well as the rat heart [56].

The best evidence for clinically relevant roles of CYP polymorphism in the development of hypertension can be found in CYP2J2 studies. The presence of a high-activity allelic variant (*CYP2J2*7*) is associated with a lower incidence of hypertension in Caucasian males. Nevertheless, this study did not demonstrate a protective effect of this allele in African-Americans [57]. Moreover, clinical observational studies till date have failed to demonstrate the clinical relevance for many other CYP polymorphisms in hypertension including the *CYP2C8*2*, *CYP2C8*3*, *CYP2C9*2* and *CYP2C9*3* alleles [57, 58]. Further association studies are needed and should include a wider range of ethnic groups and possibly other CYP isoforms.

Renovascular diseases

The role of 20-HETE and EETs in regulating renal hemodynamics has been extensively studied. 20-HETE is a powerful vasoconstrictor and an inhibitor of sodium transport in the thick ascending loop of Henle and proximal tubule, whereas EETs dilate small arterioles and block sodium transport in the collecting duct and proximal tubule [51]. 20-HETE regulates renal vascular tone by activating protein kinase C (PKC), MAPKs, tyrosine kinase and Rho kinase pathways. These lead to depolarization of vascular smooth muscle cells secondary to the blockade of BK channel which is associated with Ca^{2+} entry [59]. The upregulation of 20-HETE formationin the renal microcirculation will therefore enhance nephrovascular resistance, leading to decrease in the glomerular filtration rate and capillary pressure. The pressure in the postglomerular circulation is at the same time lowered thus shifting the pressure natriuresis relationship to higher pressures [60], leading to volume retention and hypertension. EETs are known to activate cell-surface receptors to increase the cAMP levels that activate the BK channel, resulting in vasodilation. This effect is also partially due to the stimulation of protein phosphatase 2A and activation of the BK channel in preglomerular arterioles [49]. Additional actions of EETs are the activation of small and intermediate calcium-activated potassium (K_{Ca}) channels in the endothelium that alter the driving force for calcium entry, and possibly the generation and release of nitric oxide. Furthermore, EETs enhance the vasodilator response of the renal arterioles to bradykinin, acetylcholine and adenosine [49]. Besides altering the vascular tone, 20-HETE inhibits sodium-hydrogen exchanger 3-mediated sodium and water reabsorption in the proximal tubule and sodium-potassium-chloride (NKCC2) transporter in the loop of Henle [51]. On the other hand, EETs' action on tubular transport of sodium is accomplished by regulating E_{Na}Cs activity [61].

Alteration has been demonstrated in the renal formation of 20-HETE and EETs in numerous hypertensive models, and drugs targeting these pathways have been reported to modulate pathogenesis of renal injury and hypertension in preclinical studies. Renovascular disease (RVD), a form of secondary hypertension, is one of the major causes of end-stage renal failure. It is also associated with increased cardiovascular mortality [62, 63]. In RVD, the activation of the renin-angiotensin system leads to enhanced angiotensin II activity resulting in elevated 20-HETE and decreased EET levels. These altered levels contribute to vasoconstriction, vascular remodeling, increased endothelin release, extracellular matrix deposition, and enhanced glomerulosclerosis and atherogenesis [64]. In recent years, allelic polymorphisms in *CYP4A11* and *CYP4F2* and sEHs have been linked to variations in blood pressure in several human population studies and in rodent models [65, 66]. More specifically, a nucleotide change (from T to C) at position 8590 in the human *CYP4A11* that decreases the formation of 20-HETE has been linked to the development of hypertension [67]. The G421C polymorphism in the *CYP4F2* in Chinese subjects has also been linked with elevated BP [68].

20-HETE and EETs have protective actions in renal IRI and chronic kidney disease (CKD) [69, 70]. Recent findings indicate that 20-HETE deficiency impairs vascular responses of tubular arterioles [71], raises pressure in postglomerular circulation, and increases permeability of the glomerulus to albumin, leading to the development of proteinuria and renal injury [72]. The renoprotective effect of EETs is evidenced from the fact that chronic administration of an sHE inhibitor protected against proteinuria and glomerular injury in angiotensin II and deoxycorti-

costerone acetate (DOCA)-salt hypertensive rats and mice [49]. The sEH knockout mice had lowered renal inflammation, glomerular injury, and the BP after the induction of hypertension with DOCA-salt or angiotensin II [49]. Animal studies have demonstrated the role of 20-HETE in renal IRI. A study demonstrated that a 20-HETE mimetic protected the injury induced by bilateral renal IRI by preventing the delayed postischemic fall in medullary blood flow that occurs after reperfusion [73]. Another study showed that pretreatment with a 20-HETE inhibitor protected against renal ischemia injury in a uninephrectomized model designed to mimic renal transplant injury [74].

More recent studies have implicated the involvement of 20-HETE in the pathogenesis of polycystic kidney disease (PKD) [75, 76]. Renal production of 20-HETE was elevated in a well-characterized model of PKD and blocking the formation of 20-HETE decreased cystic disease [75]. These findings suggest that 20-HETE is a new biomarker and a therapeutic target for PKD.

Development of therapeutic strategies in atherosclerotic and cardiovascular diseases

Cyclooxygenase inhibitors and thromboxane modulators in atherosclerosis

Consistent evidence supports the importance of COX-derived eicosanoids in atherogenesis as well as the benefit of COX inhibition in decreasing atherosclerosis [77]. The use of aspirin, a COX-1 inhibitor, for secondary prevention of atherosclerotic cerebrovascular and cardiovascular diseases is now firmly established, and its therapeutic effect is attributed to its irreversible inhibition of platelet COX-1 activation and subsequent TxA_2 biosynthesis [78]. The activation of TP receptor-mediated endothelial and inflammatory responses, as seen in atherosclerotic lesion formation, is thus prevented.

While aspirin inhibits TxA_2 formation, it does not affect the production of other TP receptor ligands such as hydroxyeicosanoic acid and isoprostanoids, which are also important in preserving atherosclerotic vascular injury. The blockade of TxA_2 formation by aspirin is also limited to platelets, thus TP receptor antagonism could additionally inhibit the effect of macrophage-derived TxA_2 that contributes to the inflammatory status, therefore offering additional therapeutic advantage. Furthermore, a direct inhibition of TP-receptor may exert a superior antiplatelet effect than aspirin in high-risk conditions characterized by increased synthesis of prostanoids such as diabetes [79]. These lines of evidence have led to a new research area looking into TP receptor antagonism as a viable therapeutic strategy in treating atherosclerotic diseases. The possibility of combining antiplatelet activity (using aspirin) with an antiatherosclerotic effect *via* selective TP receptor inhibition therefore represents an attractive therapeutic intervention because tackling decreased *via* multiple mechanisms would presumably treat diseases more effectively than relying on just one.

Beneficial effects of TP receptor blockade have been demonstrated through many animal studies. Daltroban, a TP receptor antagonist, has been shown to decrease the progression of atherosclerosis in cholesterol-fed rabbits [80]. Another antagonist terutroban exhibited antithrombotic action by inhibiting platelet and fibrinogen deposition in a porcine model [81, 82]. The regression of atherosclerotic lesions has been observed in a rabbit model of advanced atherosclerosis following 6 months of terutroban treatment [83]. In an earlier study, terutroban was found to decrease serum levels of TxB_2, the stable metabolite of TxA_2, and significantly decreased aortic root-lesion formation and serum levels of ICAM-1 in mice [84].

While antiatherosclerotic effects of TP receptor inhibition have been clearly demonstrated in pre-clinical models of disease, the clinical findings in patients have however not been convincing. In the Prevention of cerebrovascular and cardiovascular Events of ischemic origin with teRutroban in patients with a history oF ischemic strOke or tRansient ischeMic attack (PERFORM) study conducted recently, terutroban was shown not to be superior to aspirin in preventing stroke among patients with ischemic cerebrovascular disease [85, 86]. This seems to suggest that the pathophysiology of ischemic stroke and coronary events are somewhat complicated and more studies comparing other novel antiplatelet drugs with aspirin in recurrent stroke prevention are warranted. Furthermore, prevention approaches may require more vigorous and multi-pronged intervention, and the selection of appropriate thera-

py should also be individualized according to patient comorbidities, tolerability to drugs and efficacy of the selected agents.

EET modulators in cardiovascular diseases

In consideration of the important role of sEH in EET bio-inactivation, various selective and potent sEH inhibitors have been developed and evaluated in recent years by both academic and pharmaceutical industry laboratories and offer considerable promise as a therapeutic strategy for cardiovascular diseases [87, 88]. Another pharmacological strategy pursued actively by researchers relies on increasing CYP-derived EETs *via* the administration of EET analogs as increased endothelial EET level has proven to confer various cardioprotective effects as described in the previous sections of this paper.

sEH inhibitors have shown efficacy as antihypertensive agents in the angiotensin II-dependent animal model [54, 89]. In addition to their antihypertensive effect, sEH inhibitors were able to improve endothelial function and decrease hypertension-induced renal injury and cardiac hypertrophy or dysfunction [90]. Therefore, they have demonstrated beneficial effects in various cardiovascular disorders, including IRI and heart failure [89, 91]. A good example is AR9281 [1-(1-acetyl-piperidin-4-yl)-3-adamantan-1-yl-urea], a synthetic sEH inhibitor, that has undergone phase I and II clinical trials [92, 93]. AR9281 showed a good safety profile and it directly and dose-dependently inhibited blood sEH activity in healthy subjects. Its efficacy in patients with hypertension and type 2 diabetes has also been evaluated. Recently, the efficacy of EET analogs in the treatment of hypertension has also been demonstrated. For instance, an 11,12-EET analog was shown to lower blood pressure in spontaneously hypertensive rats [3].

Potential adverse effect of pharmacological modulation of EET biosynthesis and inactivation pathways is an important consideration during the development of EET modulators. There is evidence that sEH inhibitors can exacerbate hypoxic pulmonary vasoconstriction and hypoxia, attenuate vascular responsiveness in the lung, and induce pulmonary vascular remodelling [94]. In addition, due to their inhibitory effect on platelet aggregation [95, 96], EETs can hyperpolarize platelets and inactivate them by inhibiting adhesion molecule expression, potentially leading to bleeding and hemorrhaging in patients taking sEH inhibitors. Another adverse effect of great concern is that sEH inhibitors and EET analogs can promote angiogenesis, resulting in the acceleration of tumorigenesis [54, 97]. In fact, Panigrahy *et al.* [98] have recently demonstrated that EETs have a potent stimulatory effect on primary tumor growth and angiogenesis. Moreover, elevated EETs enhanced tumorigenesis and multiorgan metastasis in different mouse models of cancer [99]. Therefore, further research in this area should include prudent medicinal chemistry approach in designing specific EET modulators with a higher degree of target tissue specificity and decreased angiogenic and mitogenic properties. For instance, a new series of sEH inhibitors have been modified to decrease their proangiogenic properties while maintaining potent and selective sEH inhibition [100]. In fact, data suggest that EET-mediated cancer cell proliferation is regioisomer-specific [101]. Thus, sEH inhibitors and EET analogs devoid of tumorigenesis property can potentially be developed using isomer-guided drug design approach.

Conclusion

Over the past 20 years, numerous works have indicated that CYP enzymes generate a spectrum of bioactive lipid mediators from endogenous substrates. Most is known about the functional role of CYP5A1 and CYP8A1 in atherogenesis and the cardiovascular actions of members belonging to CYP2C, CYP2J and CYP4 families. Both CYP5A1 and CYP8A1 provide a Yin-Yang system during the normal process of blood coagulation *via* their gene products TxA_2 and PGI_2. The CYP2C and CYP2J epoxygenases generate EETs, which possess cardioprotective properties. The CYP4A and CYP4F ω-hydroxylases generate 20-HETE that is pivotal to renal microvascular function. The role of these CYPs in human diseases is evidenced from the association of genetic polymorphism of some of these CYP members with vascular pathology and cardiovascular disorders. Potentially viable clinical therapeutic strategies are emerging by the way of enhancing EET availability *via* EET analogs or sEH inhibitors in the clinical management of

patients at high cardiovascular risk. Similarly, blocking TxA_2 action using TP receptor antagonists has demonstrated promising antiatherosclerotic effects. The use of these novel classes of agents however is currently limited due to potential adverse effects including angiogenesis and tumorigenesis. Appropriate drug design studies should be performed to identify promising agents with better safety profile before they can be considered for further clinical trials and eventually clinical use in humans.

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Disclosure of conflict of interest

None.

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