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Molecular mechanisms and cell signaling of 20-hydroxyeicosatetraenoic acid in vascular pathophysiology

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1. ABSTRACT

Cytochrome P450s enzymes catalyze the metabolism of arachidonic acid to epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acid and hydroxyeicosatetraeonic acid (HETEs). 20-HETE is a vasoconstrictor that depolarizes vascular smooth muscle cells by blocking K^+ channels. EETs serve as endothelial derived hyperpolarizing factors. Inhibition of the formation of 20-HETE impairs the myogenic response and autoregulation of renal and cerebral blood flow. Changes in the formation of EETs and 20-HETE have been reported in hypertension and drugs that target these pathways alter blood pressure in animal models. Sequence variants in CYP4A11 and CYP4F2 that produce 20-HETE, UDP-glucuronosyl transferase involved in the biotransformation of 20-HETE and soluble epoxide hydrolase that inactivates EETs are associated with hypertension in human studies. 20-HETE contributes to the regulation of vascular hypertrophy, restenosis, angiogenesis and inflammation. It also promotes endothelial dysfunction and contributes to cerebral vasospasm and ischemia-reperfusion injury in the brain, kidney and heart. This review will focus on the role of 20-HETE in vascular dysfunction, inflammation, ischemic and hemorrhagic stroke and cardiac and renal ischemia reperfusion injury.

2. INTRODUCTION

Eicosanoids are 20-carbon fatty acids produced from the metabolism of arachidonic acid (AA) via cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) monooxygenases (Figure 1). It has long been recognized that COX and LOX metabolize AA to 5-, 12- and 15-hydroxyeicosatetraenoic acid (HETE), leukotrienes, prostaglandins (PG), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) which are involved in the regulation of fever, inflammation, cancer, vascular tone, central nervous system and airway

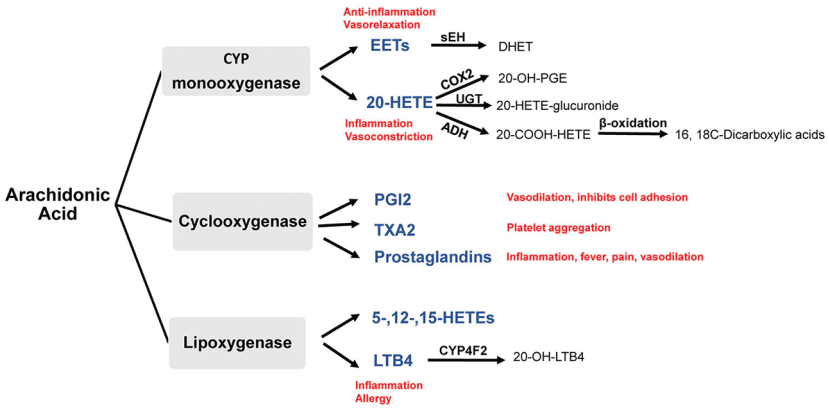

Figure 1. Pathways for the metabolism of arachidonic acid. Arachidonic acid is metabolized by enzymes of the cytochrome P450 (CYP) monooxygenase, cyclooxygenase and lipoxygenase pathways to epoxyeicosatrienoic acid (EETs), 20-hydroxytetraenoic acid (20-HETE), prostacyclin (PGI_2), thromboxane A2 (TXA_2), Prostaglandins (PGE_2 and PGF_{2a}), 5-, 12-, and 15-hydroxyeicosatetraenoic acids (HETEs) and leukotrienes. EETs are metabolized by soluble epoxide hydrolase ($_SEH$) to the corresponding dihydroxyeicosatrienoic acids (DHETEs). 20-HETE is metabolized by cyclooxygenase 2 (COX_2) to 20 hydroxy-prostaglandins (20-OH-PGE); conjugated by uridine 5'-diphosphoglucuronosyltransferase (UGT) in the liver to form a glucuronide and metabolized by alcohol dehydrogenase (ADH) to 20-carboxy-hydroxyeicosatetraenoic acid (20-COOH-HETE that is converted to shorter chain dicarboxylic acids by β-oxidation. Leukotrienes are metabolized by CYP4F2 enzyme to a less active 20-hydroxy metabolite (20-OH-LTB_4).

responses (1-4). The third pathway for the metabolism of AA by CYP enzymes was initially thought to only be involved in the hepatic metabolism of fatty acids (5, 6). However, as summarized in Figure 1, we now know that AA is metabolized by CYP enzymes in a wide variety of tissues to epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acids (DiHETEs) and 20-HETE which contribute to the regulation of renal, cerebral, pulmonary, vascular and cardiac function (7-18), as well as vascular hypertrophy and inflammation (19-24).

Cytochrome P450-dependent monooxygenases are heme-containing enzymes that catalyze NADPH-dependent metabolism of a wide variety of substrates. Enzymes of the CYP2C and 2J families metabolize AA to EETs that are subsequently inactivated by soluble and microsomal epoxide hydroxylases (sEH and mEH) to the corresponding DiHETEs (11, 25, 26). Enzymes of the 4A and 4F families catalyze the ω-hydroxylation of AA to produce 20-HETE (11, 25, 27) (Figure 1). The isoforms that produce 20-HETE in man are CYP4A11, 4A22, 4F2 and 4F3 (28-31). CYP4F2 exhibits the highest activity followed by 4A11 (28-30). CYP4F2 is expressed in the kidney and CYP4F3 is expressed in polymorphonuclear leukocytes (PMNs) (32). CYP4A1, 4A2, 4A3 and 4A8 are the isoforms that produce 20-HETE in rats. They are expressed in the liver, kidney, brain and blood vessels (27, 33-35). Enzymes of the CYP4F family (CYP4F1, 4F4, 4F5, 4F6) are also expressed in rats. (36 -39) CYP4F5 and 4F6 enzymes catalyze the omega-1 and omega-2 hydroxylation of leukotrienes (40). They produce 17-, 18- and 19-HETE rather than 20-HETE when incubated with AA. CYP4A12a is the only isoform that produces 20-HETE in mice (41, 42). CYP4A10 and 4A14 are also expressed in mice but they do not metabolize AA, rather catalyze the omega-hydroxylation of short chain saturated fatty acids (42). 20-HETE is inactivated via metabolism by COX_2 to 20-hydroxy-PGE_1 (8, 43, 44) and alcohol dehydrogenase to 20-carboxy-eicosatetraenoic acid (20-COOH-HETE) followed by metabolism by beta-oxidation to 18- and 16-carbon dicarboxylic acids (25, 45). Circulating 20-HETE is conjugated in the liver by uridine 5'-diphosphoglucuronosyltransferase (UGT) to a glucuronide (46, 47) that is filtered and excreted in the urine (48).

The expression of CYP4A protein is greater in arterioles than in large arteries, and the production of 20-HETE is inversely proportional to blood vessel diameter (49). 20-HETE is largely synthesized by vascular smooth muscle cells (VSMC) in arterioles (13, 25, 33, 50-52). The exceptions are that it has been reported to be produced in the pulmonary endothelium, (18, 53, 54), in the systemic vascular endothelial cells in animals transduced with a CYP4A2 adenovirus (55) or lentivirus (56), and in dihydrotestosterone induced hypertensive rats (57-59). 20-HETE is also produced in the neurons (60) and astrocytes in the brain (61), cardiomyocytes (62, 63), pulmonary endothelium, hepatocytes (16, 64, 65), renal proximal tubular, thick ascending loop of Henle (TALH) cells and the glomerulus in the kidney (66-68), and in leukocytes and platelets (14, 69, 70). EETs are produced by endothelial cells in the renal (26), cerebral (13, 71), pulmonary (72, 73) and coronary vascular beds (7, 74, 75), in the proximal tubule and collecting duct of the kidney, astrocytes in the brain (76-78), cardiac myocytes (79, 80), enterocytes in the gastrointestinal tract and islets of Langerhans cells in the pancreas (81, 82).

20-HETE plays an important role in regulation of a wide variety of normal physiological functions

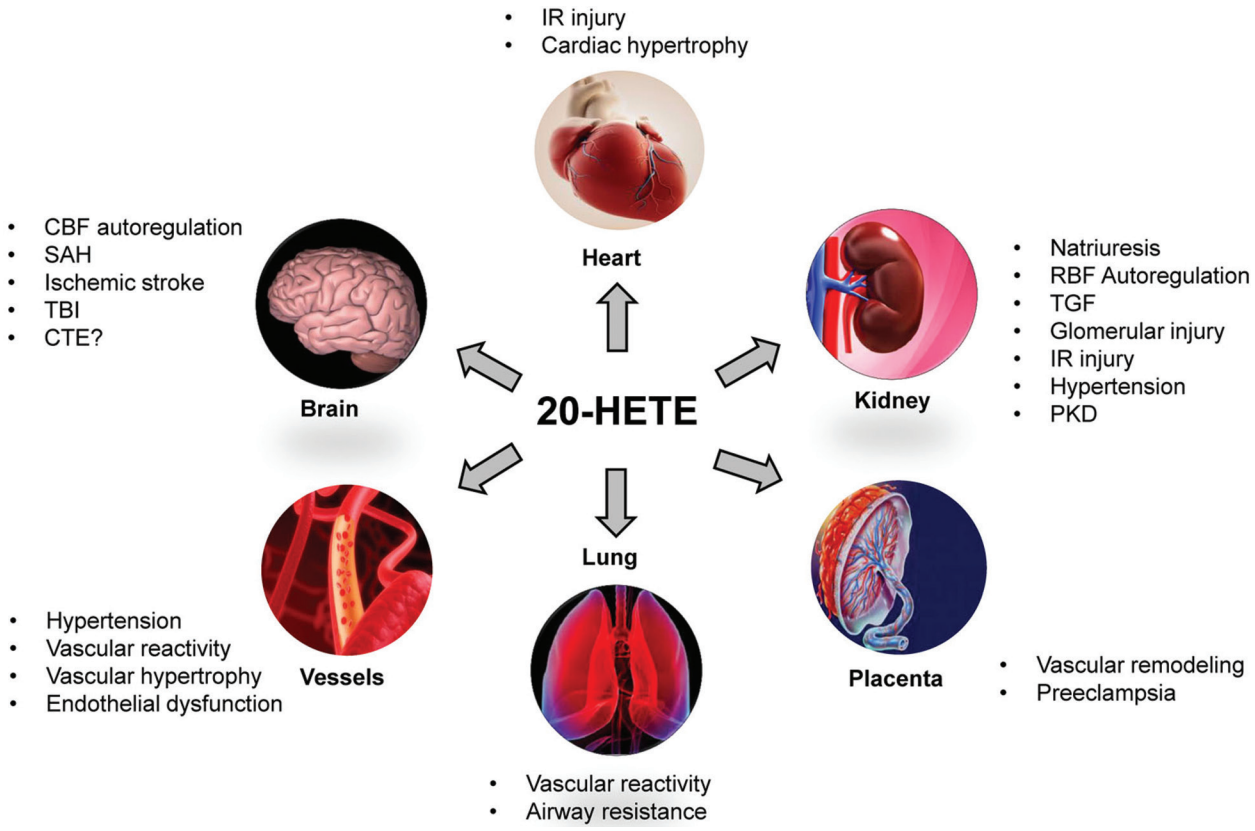

Figure 2. Diverse physiological and pathological effects of 20-HETE. 20-HETE is a potent vasoconstrictor in the renal and cerebral circulation that potentiates the vasoconstrictor response to Ang II, endothelin and other Gq dependent vasoactive agents. It promotes vascular hypertrophy, endothelial dysfunction, vascular restenosis, angiogenesis and vascular inflammation. In blood, 20-HETE inhibits platelet aggregation. In the lung, 20-HETE is an endothelial dependent dilator that reduces pulmonary vascular resistance and lowers airway resistance. 20-HETE plays an important role in the myogenic and tubuloglomerular feedback responses of the renal afferent arteriole. It also inhibits sodium transport in the proximal tubule and thick ascending loop of Henle. Deficiencies in the renal formation of 20-HETE are associated with the development of salt-sensitive forms of hypertension and renal ischemia reperfusion injury. Overproduction of 20-HETE is linked to the development of polycystic kidney disease. 20-HETE plays an important role in the regulation of cerebral vascular tone and elevations in the production of 20-HETE is associated with cerebral vasospasm following subarachnoid hemorrhage and infarct size following ischemic injury. In the heart, elevations in 20-HETE promote the development of cardiac hypertrophy and infarct size following cardiac ischemia.

and has been implicated in the pathogenesis of a variety of disease conditions. A summary of these effects is presented in Figure 2. 20-HETE is a potent vasoconstrictor that potentiates the response to angiotensin II (ANG II), endothelin, and ATP. It promotes vascular hypertrophy, endothelial dysfunction, vascular restenosis, angiogenesis, inflammation and apoptosis. In contrast, EETs are vasodilators that have been identified as endothelial-derived hyperpolarizing factors that mediate the response to acetylcholine and bradykinin in the presence of nitric oxide (NO) synthase and COX inhibitors (7, 25, 26). In blood, 20-HETE inhibits platelet aggregation (70). In the lung, 20-HETE contributes to the regulation of airway resistance and pulmonary vascular tone. 20-HETE has been implicated in vascular remodeling in the placenta and with the development of preeclampsia. In the renal vasculature, 20-HETE plays a critical role in the myogenic response of the afferent arteriole (Af-Art), autoregulation of renal blood flow (RBF) and tubuloglomerular feedback (TGF) responses. At the level of the renal tubules, it inhibits Na^+, K^+-ATPase (83) and sodium transport in the proximal tubule and TALH (11, 25, 84, 85). EETs are also produced in the renal proximal tubule and inhibit sodium transport. In the collecting duct, they modulate the activity of the epithelial sodium channel (25, 26). Changes in the renal production of 20-HETE have been implicated in the development of acute kidney injury (AKI) and chronic kidney disease (CKD), polycystic kidney disease (PKD) (86-88) and hypertension. Changes in the renal and/or vascular formation of EETs and 20-HETE have been reported in hypertension and drugs that target these pathways have altered blood pressure in various animal models (27, 41, 89-97). In the heart, 20-HETE plays an important role in ischemic injury and cardiac hypertrophy. It is also produced in the cerebral vasculature, glial cells and neurons, and is associated with ischemic and hemorrhagic stroke and brain injury. Recent human studies have indicated that sequence variants in CYP4A11 (28, 31, 98-110) and CYP4F2 (99, 110-115) that produce 20-HETE as

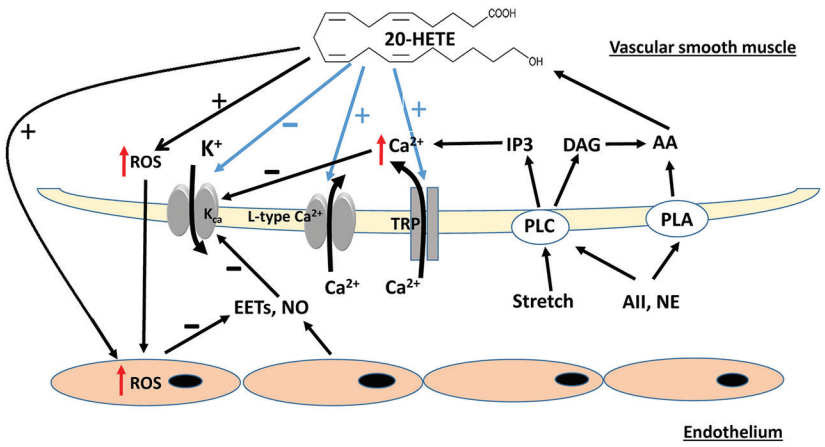

Figure 3. Mechanisms contributing to the vasoconstrictor action of 20-HETE. Increases in vascular stretch or administration of Gq coupled vasoconstrictor agonists activate phospholipase A (PLA) secondary to inositol triphosphate (IP3) mediated release of intracellular Ca^{2+}. PLA_2 catalyzes the releases of arachidonic acid from membrane phospholipids and stimulates the formation of 20-hydroxyeicosatetraenoic acid (20-HETE). 20-HETE activates phosphokinase C to inhibit activity of the large conductance, calcium activated potassium channel (K_{Ca}) to depolarize the cell and increases the activities of the L-type calcium and TRPC6 channels to promote Ca^{2+} entry.

well as UGT that is involved in the biotransformation of 20-HETE (46), are all associated with hypertension and/or stroke.

There has been considerable interest in the role of 20-HETE in the regulation of vascular tone, renal function and blood pressure (25, 83-85, 116). This has been the subject of several recent and definitive reviews (11, 26, 117-121). The role of 20-HETE in ischemic and hemorrhagic stroke (122-125), acute renal failure (126, 127), toxemia of pregnancy (128), hepatorenal syndrome (129), vascular restenosis (23), angiogenesis (24), cardiac hypertrophy (130) and ischemia reperfusion (IR) injury (131), as well as shock (132, 133) is less well known and has received little attention in the literature. This review will focus on the emerging pathogenic role of 20-HETE as an important mediator in vascular dysfunction, inflammation in a variety of diseases.

3. 20-HETE IN THE CONTROL OF VASCULAR FUNCTION

3.1. 20-HETE and the regulation of vascular smooth muscle tone

Cytochrome P450 eicosanoids play an important role in the regulation of vascular function (11, 25). 20-HETE is produced by VSMC in the renal and cerebral microcirculation (11, 25, 26). As summarized in Figure 3, 20-HETE largely serves as an autocrine second messenger similar to diacylglycerol (DAG). There is some evidence that 20-HETE, produced in astrocytes (61, 134) and the vascular endothelium, can also act as a paracrine factor to alter vascular function (56). 20-HETE increases vascular tone by activating protein kinase C (PKC), mitogen-activated protein kinases (MAPK), tyrosine kinase and Rho kinase which promotes Ca^{2+} influx by depolarizing the cell secondary to blockade of the large conductance calcium sensitive K^+ (BK) channel (13, 25, 52, 119, 135). Activation of PKC also increases the conductance of L-type Ca^{2+} channels (136) and activates the transient receptor potential canonical (TRPC6) and transient receptor potential vanilloid 1 (TRPV1) channels (137-139).

Elevations in transmural pressure increase the production of 20-HETE in cerebral arteries (140). Inhibition of the synthesis of 20-HETE impair the myogenic response of renal and cerebral arteries *in vitro* (11, 25, 140-142) and autoregulation of renal and cerebral blood flow (CBF) *in vivo* (11, 140, 143). 20-HETE also modulates TGF response mediated constriction of the renal Af-art (141, 144, 145) by potentiating the vasoconstrictor response of adenosine released by the macula densa (141). The formation of 20-HETE in the renal and cerebral vasculature is reduced in Dahl salt-sensitive rats (Dahl SS). These rats exhibit impaired myogenic responses in the renal Af-art and middle cerebral artery (MCA) and autoregulation of RBF and CBF (27, 33, 142, 144, 146). Transfer of the CYP4A genes responsible for the formation of 20-HETE from Brown Norway (BN) to the Dahl SS rat in a chromosome 5 consomic strain or upregulation of the expression of CYP4A1 in a transgenic Dahl SS rat restores the production of 20-HETE. This transfer rescues the myogenic response of the Af-art and autoregulation of RBF and CBF and protects the kidney and brain from hypertension induced injury (27, 33, 144, 146, 147).

In contrast to the vasoconstrictor actions of 20-HETE seen in most vascular beds, 20-HETE dilates bronchiole smooth muscle and the pulmonary artery of humans and experimental animals (18, 53, 148, 149).

CYP4A protein is expressed in both VSMC and the endothelium in the pulmonary artery (149). The vasodilator response to 20-HETE in rat pulmonary arteries is mediated by the release of NO and/or carbon monoxide (CO) by the endothelium secondly to an increase in intracellular calcium and activation of eNOS (54, 148). 20-HETE has also been reported to relax human pulmonary arteries and bovine coronary arteries pre-contracted with a thromboxane-mimetic by stimulating the synthesis and release of prostacyclin (53, 150).

The second messenger systems in VSMC activated by 20-HETE resembles the responses produced by ANG II and other Gq-protein coupled vasoconstrictors, and by PGE_2, PGF_2a and thromboxane acting on their respective receptors to promote vasoconstriction. Thus, there is considerable interest in isolating a 20-HETE receptor and targeting it for drug development. The strongest evidence favoring the existence of a membrane bound 20-HETE receptor is that structure activity studies have indicated that 19-, 15- and other HETEs and several 20-HETE analogs are competitive antagonists of its vasoconstrictor response (151, 152). The existence of inhibitory analogs indicates that there is likely a specific binding site that mediates the action of 20-HETE, but it remains unclear whether 20-HETE binds directly to PKC and other intracellular kinases like DAG or it acts on a membrane bound G protein, MAPK or tyrosine kinase receptor. The search for the receptor has also been made more difficult since 20-HETE is more lipid soluble than other lipid mediators and is avidly taken up by cells, esterified into membrane phospholipids and is highly protein bound. Some of these effects are inhibited by other fatty acids and structural analogs making it difficult to assess specific binding. As such, a membrane bound receptor has yet to be identified, but there are several groups working in this area and a receptor is expected to be identified and isolated in the next few years.

Several compounds have been developed for studying the role of 20-HETE. Originally, 17-octadecynoic acid (17-ODYA) was thought to be a specific suicide substrate inhibitor that blocks the formation of 20-HETE (153), but this compound also inhibits the formation of EETs (154). Dibromo-dodecenyl-methylsulfimide (DDMS) is a more selective inhibitor (155, 156). N-hydroxy-N'-(4-butyl-2 methylphenyl) formamidine (HET0016), and N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide (TS-011) are very potent (EC_{50} <10 nM) competitive inhibitors of the formation of 20-HETE (116, 157-159). They are highly selective when given at concentrations of 0.1 and 1 micromolar, but they will inhibit the formation of EETs at concentrations of 10 micromolar or higher. HET0016 and TS011 can be used to block the formation of 20-HETE *in vivo*. The effective dose is 1 mg/kg and it can be given *i.v., i.m. or s.c*. Chronically, these inhibitors are difficult to use as they have a short half-life (<1 hr) and have to be continuously infused or given at high dose (10 mg/kg) at least twice a day (160, 161). Since they are competitive inhibitors, the tissue concentrations of the drugs fall during homogenization. Thus, it is difficult to demonstrate that they inhibit 20-HETE production. However, it is possible to show that they reduce tissue concentrations of 20-HETE. 20-HETE analogs, 20-hydroxyeicosa-6(Z), 15(Z)-dienoic acid (6, 15−20-HEDE, WIT002) and N-(20-hydroxyeicosa-6(Z), 15(Z)-dienoyl) glycine (6, 15−20-HEDGE) have been found to antagonize the vasoconstrictor actions of 20-HETE (151, 152). The effective concentrations of these drugs *in vitro* are 1-10 micromolar. The half-life of 20-HETE agonists and antagonists are also quite short and they have to be given several times a day at high dose (10 mg/kg) when used *in vivo* (127). More recently, Pandey *et al*. (162) described a new water soluble antagonist of 20-HETE, *i.e*. 2,5,8,11,14,17- hexaoxanonadecan-19-yl-20-hydroxyeicosa 6(Z), 15(Z)-dienoate (20-SOLA). This compound lowered blood pressure when given in the drinking water to a 20-HETE dependent hypertensive mouse model (162, 163). Giving 20-SOLA throughout the day via the drinking water overcomes the problems associated with the need to infuse the 20-HETE antagonists. 20-SOLA may emerge as the preferred method to block the 20-HETE pathway.

3.2. 20-HETE and endothelial function

Endothelial cells play an essential role opposing platelet and white blood cell adhesion, and modulating vascular tone through the release of NO, PGs, EETs and CO. Endothelial dysfunction is associated with aging and numerous disease states, including hypertension, arteriolosclerosis, hyperlipidemia and diabetes (164). The hallmark of endothelial dysfunction is deficiency in the bioavailability of NO due to impaired NO production by the endothelium, and/or increased inactivation of NO by locally generated reactive oxygen species (ROS).

20-HETE produces endothelial dysfunction via several mechanisms as summarized in Figure 4. 20-HETE stimulates the formation of superoxide in endothelial cells by activation of extracellular signal-regulated kinases 1/2 (ERK1/2)/MAPK pathways that stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. It also uncouples eNOS activity to lower the production of NO and increase the formation of superoxide (165, 166). The decrease in the bioavailability of NO impairs the response to acetylcholine (ACH) and other endothelial dependent dilators.

The production of 20-HETE is elevated in the vasculature of hypertensive animal models with endothelial dysfunction. These include: rats treated with an adenovirus to upregulate the expression of CYP4A2 in the endothelium (55); spontaneously hypertensive rats (SHR) (96, 167-169); androgen-induced

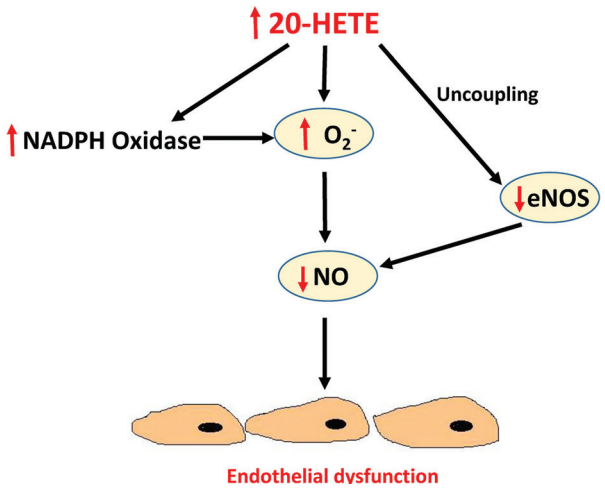

Figure 4. 20-HETE and endothelial dysfunction. Upregulation of the formation of 20-HETE in endothelial cells increases the formation of superoxide (O_2^-) by stimulating NADPH oxidase activity. It also uncouples endothelial nitric oxide synthase (eNOS) to inhibit the formation of nitric oxide (NO) and increase the formation of superoxide. The rise in superoxide levels reacts with NO to form peroxynitrite and lower the bioavailability of NO and that promotes endothelial dysfunction.

hypertensive rats (57, 58); rats treated with the immunosuppressant drug cyclosporine A which induces CYP4A expression (170); and CYP4A12 transgenic and CYP4A14 KO mice in which the production of 20-HETE is elevated (41, 171, 172). In androgen-induced hypertensive rats, the expression of the gp-91 and gp-47 phox subunits of NADPH oxidase and the production of ROS are elevated (57). Administration of inhibitor of IKB kinase prevented the increase in nuclear factor-kappa beta (NF-KB) expression and ROS production in the vasculature and impaired the vasodilator response to ACH following administration of 20-HETE (58). In SHR rats, administration of the inhibitor of the synthesis of 20-HETE, HET0016, attenuated the elevated levels of superoxide and restored the vasodilator response to ACH in the middle cerebral artery (167, 168). 20-HETE has also been reported to promote endothelial dysfunction in cultured human endothelial cells by stimulating NF-KB and the production of inflammatory cytokines (173). It plays a role in the development of atherosclerotic plaques in vessels (174) and hypertension (175, 176), both of which are associated with increased oxidative stress. More recently, Wang *et al.* (177) reported that 20-HETE may trigger thrombosis by increasing adhesion of platelets and the secretion of von Willebrand factor (vWF) in endothelial cells by activating ERK and calcium influx (177).

3.3. 20-HETE and vascular remodeling

Vascular remodeling refers to the process of altering the structure and organization of blood vessels through cell proliferation, migration, and alterations in the production of extracellular matrix (ECM). It involves activation of matrix metalloproteinases (MMPs), cytokines and inflammatory mediators (178, 179). This process involves all vascular cell types including endothelial, fibroblasts and VSMC, as well as changes in the ECM and basement membrane (180). Vascular remodeling is essential to angiogenesis and the development of new mature vessels (181), but it also is an adaptive process that responds to changes in hemodynamic and/or humoral factors and the pathophysiology of a variety of vascular disorders (180).

20-HETE has been reported to contribute to the migration and proliferation of VSMC and endothelial cells induced by ANG II (20, 182), epidermal growth factor (EGF) (22), vascular endothelial growth factor (VEGF) (24), and platelet-derived growth factor (PDGF) (183). It stimulates the secretion of the proangiogenic molecules hypoxia-inducible factor-1 alpha (HIF-1alpha), VEGF and stromal cell-derived factor-1alpha (SDF-1alpha), as well as increases the expression of the chemokine receptor type 4 (CXCR4) to promote migration to sites of neovascularization (24, 184-187). 20-HETE also uncouples eNOS to reduce the formation of NO and increase the production of superoxide in endothelial cells (165, 166).

Other studies indicate that 20-HETE enhances the apoptotic processes involved in vascular remodeling. 20-HETE inhibits the migration and invasion of human villous trophoblasts and uterine vascular smooth muscle cells (188) indicating that 20-HETE may alter uterine spiral artery remodeling and the development of preeclampsia. In the lung, 20-HETE inhibits the apoptotic response of pulmonary artery smooth muscle cells induced by the activation of the PI3K/Akt pathway by increasing the generation of ROS (189).

Vascular remodeling is a hallmark of hypertension and is associated with an increase in the media-to-lumen ratio of small arteries, which enhances vascular reactivity and peripheral resistance (179). Baumbach *et al.* (190) first coined the term “remodeling” in 1989 to describe the thickening of the vessel wall and the increase in the media-to-lumen ratio of pial arterioles in stroke-prone spontaneously hypertensive rats. The production of 20-HETE is elevated in the kidney (191, 192), and 20-HETE contributes to increased renal, cerebral and skeletal muscle vascular reactivity in SHR (167, 168, 193, 194). They exhibit profound vascular remodeling leading to the suggestion that elevated 20-HETE levels mediate hypertension induced vascular hypertrophy. In this regard, 5 alpha-dihydrotestosterone has been reported to induce the expression of vascular CYP4A12; increase the synthesis of 20-HETE and is associated with vascular hypertrophy. 20-HETE production has also been reported to contribute to vascular hypertrophy in

a CYP4A12 transgenic mouse independent of the rise in arterial pressure (172, 195). In these studies, the rise in systolic blood pressure (SBP) could be prevented by a 20-HETE antagonist, 20-HEDGE, or following blockade of the angiotensin type I receptor with losartan. However, the vascular remodeling was only prevented by 20-HEDGE (196). These authors concluded that activation of the local vascular renin-angiotensin system (RAS) contributes to 20-HETE mediated hypertension in androgen dependent models of hypertension; however, the vascular remodeling is mediated by elevated levels of 20-HETE rather than locally generated ANG II (196).

3.4. 20-HETE and restenosis

20-HETE increases the migration and proliferation of VSMC. It contributes to the actions of ANG II and a variety of growth factors (20-22, 183, 197, 198). Since NO directly binds to enzymes of the CYP4A pathway and inhibits the formation of 20-HETE (155, 199), endothelial damage that reduces the synthesis or bioavailability of NO should increase the production of 20-HETE, which contributes to vascular hypertrophy. This hypothesis is consistent with the results of previous studies indicating that the formation of 20-HETE is elevated in SHR, ANG II and androgen induced hypertensive animals that all exhibit 20-HETE dependent endothelial dysfunction and vascular remodeling (58, 167, 172, 195). Yaghini *et al.* reported that ANG II and AA increase the expression of CYP4A1 in balloon-injured (BI) rat carotid arteries and enhanced neointimal formation (200). Other investigators have found that the formation of 20-HETE in carotid arteries is elevated following balloon injury; chronic administration of HET0016 or other inhibitors of 20-HETE greatly reduced proliferation and migration of smooth muscle and neointimal formation (23, 200).

3.5. 20-HETE and angiogenesis

Sa *et al.* (201) provided the first evidence that CYP4A and 20-HETE plays a role in angiogenesis. They reported that fibroblast growth factor 2 (FGF2) activates phospholipase A_2 (PLA_2) via the p42 MAPK pathway in endothelial cells to increase the release of AA from membrane phospholipids to stimulate CYP4A to produce 20-HETE. Amaral *et al.* (202) later reported that administration of 20-HETE inhibitor blocked angiogenesis induced by chronic electrical stimulation of skeletal muscle. This was associated with upregulation of the expression of VEGF in the muscle; administration of VEGF neutralizing antibody blocked the rise in vessel density. They suggested that VEGF stimulates angiogenesis secondary to activation of PLA_2 to increase release of AA and production of 20-HETE. Jiang *et al.* (203) reported that smooth muscle specific overexpression of CYP4A1 in VSMCs promotes endothelial sprouting in cultured renal microvessels. This effect was blocked by HET0016 and the effects of HET0016 could be reversed by addition of a 20-HETE agonist. 20-HETE has been reported to increase proliferation and tube formation of human umbilical vein endothelial cells (HUVEC) cells (204). This was associated with stimulation of ROS production, the secretion of HIF-1alpha and activation of the PI3K/AKT pathway (185, 198, 205). In addition, administration of HET0016 has been reported to decrease the expression of a variety of angiogenic growth factors and inhibit growth of triple negative breast cancer tumor in mice (206). In human small cell lung cancer cell line, treatment with the 20-HETE agonist, 20-hydroxyeicosa-5(Z),14(Z)-dienoic acid (WIT003) or upregulation of the expression of CYP4A11, induced expression of VEGF and MMP-9 via PI3K or ERK pathways that significantly increased microvascular density. These effects were attenuated by 20-HETE antagonist, WIT002 or HET0016 (207).

A previous report (Figure 5) demonstrated that 20-HETE induces neovascularization in the rat cornea *in vivo.* Inhibition of the formation of 20-HETE markedly reduced the corneal neovascularization produced by VEGF and FGF (204). These authors also found that inhibition of the formation of 20-HETE blocked neovascularization of the cornea following implantation of glioblastoma cells, suggesting that 20-HETE inhibitors might prevent vascularization and proliferation of solid tumors. Subsequent studies confirmed that administration of 20-HETE inhibitor markedly reduced the vascularization and growth of rat 9L and human U251 glioma cells in the brain of nude rats (208, 209). Upregulation of the expression of CYP4A1 in U251 cells enhanced the proliferative phenotype *in vitro* and the growth of tumors *in vivo* (210). This sparked considerable interest in the use of 20-HETE inhibitors and antagonists as cancer chemotherapy agents. The findings that the expression of CYP4A/4F enzymes are elevated in human thyroid, ovarian, breast, and colon cancers (211), pancreatic adenocarcinoma (212) further support that 20-HETE may play a role in vascularization and growth of tumors. More recent studies indicate that inhibition of the synthesis of 20-HETE are effective in reducing the growth of glioblastoma, lung, prostate, renal, breast and colon cancer in experimental animals (206, 213-217).

3.6. 20-HETE and platelet aggregation

Human PMNs express CYP4F3 (32) that produces 20-HETE when incubated with AA (14, 70, 218-220). 20-HETE and 16-HETE are released from rabbit PMNs stimulated with a calcium ionophore, thrombin or platelet activating factor (14, 70, 220). 20-HETE inhibits AA- and thromboxane-induced aggregation of human platelets (70). In these studies, 20-HETE was found to inhibit the formation of thromboxane by competing for the metabolism of platelet derived AA by COX. One of the metabolites formed from 20-HETE, 20-hydroxy-thromboxane B2, may serve as a partial agonist and competitive antagonist of the thromboxane receptor. In contrast, Liu *et al.* (221) recently reported that chronic administration of COX2 inhibitors increased circulating 20-HETE levels in the plasma of mice by 120-fold, this

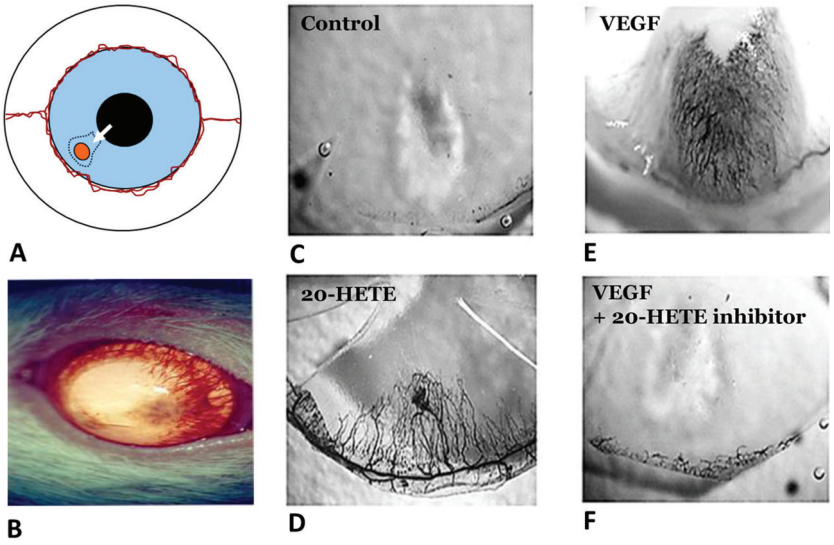

Figure 5. Angiogenic effects of 20-HETE. Panel A and B. Implantation of a pellet containing angiogenic substances in the rabbit cornea induces growth of blood vessels. Panel C and D indicates that implantation of a 20-HETE mimetic is angiogenic and increases the growth of blood vessels in the cornea of rabbits. Panels E and F indicate that implantation of VEGF, FGF and other growth factors induce angiogenesis; administration of a 20-HETE synthesis inhibitor or a 20-HETE antagonist completely blocks the angiogenic effects of VEGF and other growth factors. Figure redrawn from data presented in Chen *et al*. (180) with permission.

is associated with reduced bleeding time. They further reported that 20-HETE stimulated platelet aggregation *in vitro* in mice. The reason for the difference in the results between murine and human platelets needs to be further explored. However, Liu *et al.* (221) concluded that the adverse cardiovascular effects of COX-2 inhibitors may be due to inhibition of the metabolism of 20-HETE and subsequent increase in platelet aggregation leading to increased incidence of stroke and myocardial infarcts.

4. ROLE OF 20-HETE IN VASCULAR INFLAMMATION

Inflammation initially is a protective response in wound healing that involves activation of immune cells, release of cytokines and growth factors, and changes in vascular function and remodeling. The acute inflammatory phase is characterized by vasodilation and increased permeability of the vascular wall. Leukocytes infiltrate the injured site along with plasma containing fibrin and immunoglobulins that promote swelling. Chronic inflammation is characterized by simultaneous destruction and healing of the tissue to form an abscess or scar tissue. A cellular phase sustains the inflammatory response. Cellular-derived mediators are released including: monocyte chemoattractant protein-1 (MCP-1), interleukin 1 (IL-1), IL-6, tumor necrosis factor alpha (TNF-alpha) and interferon gamma (IFN-gamma), adhesion molecules (VCAM-1, E-selectin) and NO, all of which are associated with vascular inflammation and dysfunction.

Eicosanoids play important roles as pro- or anti-inflammatory agents. The roles of LOX or COX-derived eicosanoids in inflammation have been extensively studied (1, 2, 222, 223). Nonsteroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory action by inhibition of COX and the synthesis of prostaglandins and thromboxane. They are widely used to treat inflammation, reduce pain and fever and prevent colorectal cancer. Low dose aspirin is also one of the primary therapies to prevent platelet activation and reduce the risk of heart attack and stroke. Leukotriene inhibitors and receptor antagonists exert their anti-inflammatory effects by blocking the formation and actions of leukotrienes to promote infiltration of leukocytes and inflammation. Much less is known about the role of CYP4A and 4F enzymes in inflammation. LTB4, which plays a key role in recruitment of leukocytes, is metabolized by CYP4F2 enzymes to a less active metabolite, 20-hydroxy-LTB4. This product is further metabolized to 20-carboxy-LTB4 and then degraded by beta-oxidation (40, 224, 225). Thus, inhibition of CYP4F2 may enhance inflammation by increasing LTB4 levels. Moreover, 20-HETE has been reported to play a role in vascular inflammation by regulating the expression and activities of inflammatory mediators, cytokines (63, 173) and adhesion molecules (173, 226) in endothelial cells as well as by altering the levels of NO (54), superoxide and oxidative stress (227).

Induction of sepsis with lipopolysaccharide (LPS) is associated with activation of MEK1/ERK1/2/IKKbeta/IKB-alpha/NF-KB and MyD88/TAK1/IKKbeta/IKB-alpha/NF-KB pathways that increases the levels of inflammatory cytokines (TNF-alpha and IL-8) (133, 228). These changes, along with the associated hypotension and tachycardia, can be prevented by administration of

a stable 20-HETE mimetic, 5, 14-HEDGE. The beneficial effects of the 20-HETE agonist in sepsis were reversed by administration of a 20-HETE antagonist (133, 228). Previous studies have shown that NO binds to heme of the CYP4504A enzymes and inhibits the formation of 20-HETE (155, 199, 229-231). The protective effect of 5, 14-HEDGE in septic shock is thought to counteract the inhibition of the formation 20-HETE by NO (227). In addition, administration of 20-HETE agonist reduces the upregulation of COX-2 and gp91phox activity suggesting that 20-HETE may have a direct anti-inflammatory effect in septic shock (227). On the other hand, it is possible that this effect maybe simply due to the prevention of hypotension and tissue ischemia normally associated with sepsis.

20-HETE has been reported to promote inflammation by activating ED-1 and monocytes/ macrophages induced by acute renal IR injury in uninephrectomized Lewis rats (232). The infiltration of inflammatory cells in the kidney was attenuated by HET0016 and 6, 15-20-HEDE, and enhanced by 5, 14-20-HEDE (232). Interestingly, 20-HETE has the opposite effect and plays a protective role in bilateral renal IR injury in rats (126, 127). An anti-inflammatory effect of 20-HETE was also observed in DOCA-salt hypertensive mice treated with fenofibrate that induces the expression of CYP4A and the production of 20-HETE via stimulation of the peroxisome proliferator-activated receptors (PPAR) -alpha receptor (233).

Toth *et al.* recently reported that 20-HETE promotes cerebrovascular inflammation in SHR rats by increasing vascular production of ROS and activating NF-KB. Treatment with HET0016 significantly decreased oxidative stress in the MCA of SHR and attenuated the activation of vascular NF-KB and the expression of TNFalpha, IL-1beta and IL-6 (167). In addition to increasing cytokine production, 20-HETE stimulates the expression of adhesion molecules in endothelial cells (173, 177, 226). Elevated levels of 20-HETE promote the expression of ICAM-1 and VCAM-1 on B-lymphocytes which interact with receptors on endothelial cells (226, 234). The increased expression of the adhesion molecules enhances the adhesion of macrophages to the vessel wall and triggers a proinflammatory state.

5. 20-HETE IN STROKE AND TRAUMATIC BRAIN INJURY

Each year, 750,000 strokes occur in the United States. There are large numbers of survivors with devastating neurological symptoms that cannot return to work because of physical limitations. The annual cost to treat stroke victims is 51 billion dollars, and rising. Approximately 80% of strokes are ischemic (due to interruption of blood supply) and 20% are hemorrhagic. Recent studies have indicated that 20-HETE contributes to the development of acute and delayed vasospasm, delayed cerebral ischemia and infarct size following ischemic stroke.

5.1. 20-HETE and subarachnoid hemorrhage (SAH)

SAH commonly occurs after rupture of a cerebral aneurysm leading to subarachnoid bleeding and clot formation. It causes acute cerebral ischemia (ACI) lasting several hours due to acute cerebral vasospasm and a rise of cerebral spinal fluid pressure, followed by the later development of delayed vasospasm and cerebral ischemia (DCI) (116). The majority of deaths occurs within the first 2 days of the injury and is associated with extensive ischemic injury to the brain (235-237). The later development of DCI is a long-term prognostic factor for outcomes in both SAH and TBI (238-240). One-third of patients that develop delayed cerebral vasospasm and DCI die, one-third suffer permanent neurological damage while the remainder recovers (237, 241).

The factors that initiate the acute fall in CBF following SAH remain uncertain. The fall in CBF correlates with the amount of hemoglobin (Hb) in cerebrospinal fluid (CSF) and can be triggered by injection of clotted blood or oxyHb into CSF (242-246). There is evidence that acute vasospasm is triggered in part by scavenging of NO (237, 245) by Hb and the release of the vasoconstrictors, serotonin (5-HT), ATP and endothelin (ET) from clotting blood (237, 242, 245, 247). ET (248, 249), thromboxane (245), isoprostane, (250, 251) and 5-HT (252) levels are elevated in CSF following SAH and the responsiveness of the vasculature to these vasoconstrictors are enhanced (242, 253, 254). There is also release of AA and increased formation of COX and LOX metabolites (237). Acute cerebral vasospasm has been reported to be attenuated by blockade of ET synthesis or the ET_A receptor (255-257), blockade of the 5-HT1b receptor (252), thromboxane antagonists (258, 259),as well as inhibitors of RAS (260), Rho (261-263), MAP kinase(264), PKC (265, 266) and Src kinase (267).

Delayed vasospasm is associated with activation of PKC (243-245, 257, 265, 268), MAP (264), and Rho kinase (263), diminished K^+ channel activity and depolarization of VSM cells (269). The vascular responsiveness to ET, 5-HT and other vasoconstrictors is elevated following the development of delayed vasospasm (243, 253, 270) and there is a diminished responsiveness to NO and other endothelial dependent vasodilators (269, 271-274). The endothelial dysfunction associated with delayed cerebral vasospasm has been attributed to binding of NO to Hb and increased destruction of NO by superoxide (243, 272-274).

The results of previous studies indicate that cerebral arteries produce the potent vasoconstrictor 20-HETE, which has effects on the cerebral vascular tone that mimic those seen following SAH. The level of

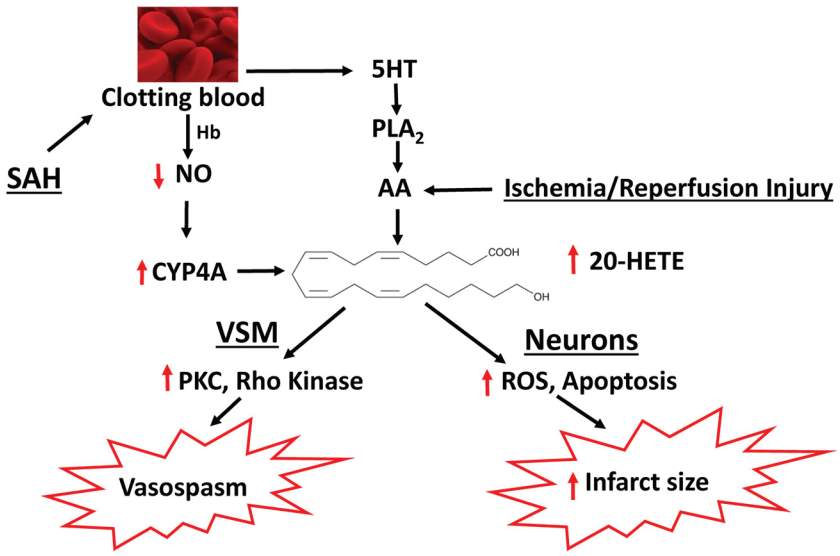

Figure 6. Role of 20-HETE in hemorrhagic and ischemic stroke. Subarachnoid hemorrhage (SAH) leads to the release of free hemoglobin (Hb) and serotonin (5-HT) from clotting blood. Serotonin activates phospholipase A_2 in the cerebral vasculature to increase the release of arachidonic acid and the formation of 20-hydroxyeicosatetraenoic acid (20-HETE). Free hemoglobin scavenges NO and the fall in NO levels increase the activity of CYP4A enzymes and the formation of 20-HETE. The rise in 20-HETE levels contributes to acute and delayed vasospasm in SAH. 20-HETE levels are also elevated in cerebral tissue, CSF and plasma following ischemic stroke. The increase in 20-HETE levels was initially thought to promote infarct size by reducing blood flow in the penumbral regions, but subsequent work indicates that 20-HETE increases oxidative stress in ischemic neurons and promotes apoptosis and cell death.

20-HETE in CSF was elevated in patients (275-278) and experimental animals following SAH (124, 279). Inhibition of the synthesis of 20-HETE prevented the acute fall in CBF following SAH in rats (122) and reversed delayed vasospasm in both dogs and rats (124, 279, 280). These findings demonstrate an important role of 20-HETE in the development of acute and delayed cerebral vasospasm, at least in experimental animal models, and suggest that inhibitors of the synthesis of 20-HETE or 20-HETE antagonists may have therapeutic potential in the treatment of these devastating conditions.

Cortical spreading ischemia (CSI) is another mechanism that contributes to ischemic damage in patients following SAH (281). It is caused by cortical spreading depolarization (CSD), which is a wave of mass neuronal depolarization after SAH that is associated with release of AA, impaired neurovascular coupling and reduced CBF (281, 282). CSD has recently been reported to be associated with an increase in 20-HETE synthesis and reduced CBF in rat model. Administration of 20-HETE inhibitor ameliorated the reduction in CBF, decreased edema and afforded neuroprotection (282, 283).

The proposed mechanism by which 20-HETE contributes to changes in CBF following SAH is presented in Figure 6. Hb that is released following SAH increases the production of 20-HETE in the cerebral vasculature by scavenging NO and CO that chronically inhibit the formation of 20-HETE. AA and vasoconstrictors (ET, ATP and 5-HT) that stimulate the formation of 20-HETE are also released by clotting blood after SAH. 20-HETE plays an important role in the development of acute vasospasm following SAH. CBF, however, rapidly recovers within 12-24 hrs following SAH. This is associated with increased production of NO and CO, both of which inhibit the formation of 20-HETE. However, NO and CO upregulates the expression of CYP4A and 4F enzymes during the recovery phase and this contributes to secondary elevation in the production of 20-HETE and delayed vasospasm after the blood surrounding cerebral arteries is cleared and NO and CO levels return to control.

5.2. 20-HETE and traumatic brain injury (TBI)

TBI with intracranial bleeding exhibits many features common with SAH. CBF autoregulation is impaired following TBI (284-287) which is associated with CSD in patients and experimental animals (288). TBI patients that exhibit impairments in autoregulation of CBF are more likely to have a worse outcome (289 292). Although the mechanisms involved are not yet completely understood, there is some evidence, that 20-HETE may contribute to the impaired autoregulation of CBF after TBI. TBI activates phospholipases, increases the release of fatty acids and AA and the formation of 20-HETE. (282, 293-296) There is increased expression of CYP4A and CYP4F in the injured area long term following TBI (225, 297). For example, the expression of CYP4A8 in the hippocampus was increased 6 hours after TBI and the expression of CYP4A1, CYP4F5 and 4F6 remained elevated for up to 7 days (297).

5.3. 20-HETE and ischemic stroke

Approximately 80% of strokes are due to thromboembolic interruption of the blood supply to the brain (298). The most common experimental models involve permanent or transient occlusion of the MCA (MCAo) of rodents (299-301). Following occlusion of a cerebral artery, there is depletion of ATP and high-energy phosphates (299, 302, 303), reduced Na^+, K^+-ATPase activity, cellular acidification (303), accumulation of intracellular Na^+ and Ca^{2+} and loss of intracellular K^+ in the ischemic core. With prolonged ischemia (2-6 hrs), there is cell swelling and rupture of intracellular organelles, loss of the integrity of mitochondrial membranes (304), release of proteolytic enzymes, and death of the neurons. This is followed by cellular necrosis and influx of inflammatory cells. Cerebral ischemia is also associated with the release of mediators that augment the degree of ischemic damage. These include: the excitatory amino acids, glycine, glutamate and aspartate (305-309), ET, dopamine (307), 5-HT and free fatty acids (310-312). The excitatory neurotransmitters contribute to cell death by increasing metabolic rate and oxygen demand, especially in the penumbra after reperfusion (313). The free fatty acids contribute to neuronal damage by serving as substrates for the formation of lipid peroxides that have detrimental effects on cellular metabolism and oxygen utilization (314-316). Other fatty acids released during ischemia, such as AA, are converted to HETEs, leukotrienes and eicosanoids (317-319) that compromise collateral flow, increase the production of free radicals (208) and recruit leukocytes in the ischemic area. The recruitment of leukocytes and swelling of the brain may also reduce flow to the injured tissue at the margins of the infarct.

There is considerable evidence that 20-HETE plays an important role in determining infarct size following ischemic stroke. The levels of 20-HETE are elevated in cerebral tissue in rat (123, 320) and in the plasma of patients (321) and rats (50) following ischemic stroke. Administration of 20-HETE synthesis inhibitors markedly reduces infarct size and improves neurological outcomes following focal cerebral ischemia in rats (50, 116, 158, 168, 322, 323). The production of 20-HETE is elevated in the cerebral vasculature in SHR stroke-prone rats, and they exhibit an enhanced sensitivity to ischemic stroke (168) along with 20-HETE dependent oxidative stress and endothelial dysfunction in the cerebral circulation. Mutations in CYP4F2 and CYP4A11 have been linked to an increase in the incidence of ischemic stroke in Chinese, Swedish and Japanese populations (102, 113-115). Genetic variants in CYP450 isoforms are associated with plaque stability in patients with ischemic stroke (125).

The mechanism underlying the neuroprotective effects of blocking 20-HETE synthesis following cerebral ischemia and reperfusion was originally thought to be due to blockade of its vasoconstrictor effects. Thus, blockade of the formation and release of 20-HETE may improve collateral blood flow and oxygen delivery to the penumbra and reduce infarct size. However, subsequent studies indicated that inhibition of 20-HETE reduce infarct size, but had no effect on CBF in the ischemic core or surrounding tissue (324). This leads to speculation that perhaps other mechanisms might be involved. For example, blockade of the synthesis of 20-HETE might reduce the recruitment of PMNs, inflammation and oxidative stress in the at risk tissue or have direct neuroprotective actions. In support of this later possibility, Renic *et al.* (60) reported that inhibition of 20-HETE markedly reduced necrosis and apoptosis of neonatal brain slices subjected to oxygen and glucose deprivation. The beneficial effect was associated with a reduction in superoxide production and activation of caspase-3. 20-HETE activates a host of cell signaling pathways, such as tyrosine kinase, MAP kinase, PKC, RAS and Rho (20, 325-329) involved in apoptosis and cell death. Thus, blockade of the formation of 20-HETE might reduce oxygen consumption or interfere with the signal transduction cascade leading to cell death. Moreover, 20-HETE also inhibits Na^+, K^+-ATPase (83, 330). It may contribute to the accumulation of Na^+, depolarization of neurons that triggers Ca^{2+} influx, and the release of excitatory amino acids, fatty acids, eicosanoids and other mediators from the brain after reperfusion.

6. ROLE OF 20-HETE IN RENAL AND CARDIAC ISCHEMIA REPERFUSION (IR) INJURY

6.1. 20-HETE and renal ischemia reperfusion injury

Acute kidney injury is a common complication following surgery and several medical conditions that significantly increases morbidity and mortality (331, 332). Renal IR injury is the most common cause of AKI (332, 333). AA is released from membrane phospholipids in response to ischemia and can be metabolized to 20-HETE (334). Indeed, we recently reported that 20-HETE levels are markedly increased in the renal outer medulla following IR (126, 127). In the kidney, 20-HETE enhances vascular tone and reduces tubular sodium transport (8, 11, 25, 84, 85). 20-HETE increases vascular responsiveness to vasoconstrictors and would be expected to reduce tissue blood flow and exacerbate IR injury as has been reported both in the heart and the brain (50, 334). Several reports have addressed the role of 20-HETE on renal IR injury. Regner *et al*. demonstrated that blockade of 20-HETE with HET0016 increased bilateral renal IR injury; administration of 20-HETE agonists (5,14-20-HEDGE and 5,14-20-HEDE) reduced bilateral renal IR injury by preventing the secondary fall in medullary blood flow and prolonged renal ischemia (126, 127). On the

other hand, Hoff *et al*. (232) reported that 20-HETE inhibitor (HET0016), or a 20-HETE antagonist (6, 15-20-HEDE), protected the kidney against renal IR following uninephrectomy. The reasons for the divergent results remain unresolved, but appear due to differences in the experimental models in these two studies since in a follow up study Roman *et al.* (335) confirmed that blockade of the formation of 20-HETE opposes renal IR injury in bilateral ischemic model, but enhances injury in the uninephrectomized rats. A recent study in Dahl SS rats, which have a genetic deficiency in the expression of CYP4A enzymes and the formation of 20-HETE relative to other strains (27, 336), indicated that they are much more susceptible to renal IR injury (337). Transferring of the CYP4A genes on chromosome 5 from BN rats into the Dahl SS genetic background in a consomic strain normalized the production of 20-HETE and the susceptibility to renal IR injury (126).

20-HETE has differing effects on renal tubular and vascular function, thus the mechanism of its renoprotective effects in IR injury remains to be determined. It activates a number of signaling pathways that could promote or attenuate renal IR injury. In this regard, 20-HETE is a potent constrictor of preglomerular arterioles that may exacerbate reductions in RBF after initial ischemic injury, leading to secondary injury (25). Prolonged exposure to high concentrations of 20-HETE may also potentiate renal IR injury by increasing the generation of reactive oxygen species both by stimulating of NADPH oxidase directly and/or through uncoupling of nitric oxide synthase (338). On the other hand, 20-HETE may oppose renal IR injury since it has been shown to prevent the secondary fall in medullary blood flow and the prolonged medullary ischemia (126, 127). It also inhibits sodium transport and could reduce oxygen consumption in this region of the kidney (8, 25, 84, 85, 126, 339). In addition, 20-HETE and 5,14-20-HEDE activates the Raf–MEK–ERK and phosphatidylinositol-3′-kinase/AKT pathways in renal tubular epithelial cells (340). Activation of the AKT pathway has been reported to enhance renal epithelial-cell survival in models of renal IR injury (341, 342).

6.2. 20-HETE and allograft function in kidney transplantation

IR is an unavoidable process in kidney transplantation. It determines both early and long-term allograft function that affects morbidity and mortality. Previous studies have suggested that vascular dysfunction, tubular injury, and inflammation all contribute to renal IR injury after kidney transplantation (343-347). Following cold ischemia, intracellular Ca^{2+} concentration increases which activates PLA_2 to promote the release of AA from membrane phospholipid pools. As discussed earlier, AA can be metabolized into prostaglandins, leukotrienes and 5-, 12-, 15-HETE by COX and LOX, and 20-HETE and EETs via cytochrome P450 pathways (25). Dolegowska *et al*. (348) reported that during the first 5 minutes following allograft reperfusion, the concentration of 20-HETE in a blood sample is significantly elevated in patients with good graft function (serum creatinine less than 3 mg/dl in 5 days). It was unchanged in patients with delayed graft function (serum creatinine greater than 3 mg/dl); and very low in patients with poor graft function that requires dialysis. Moreover, perioperative 20-HETE levels in plasma are strongly associated with improved graft function following renal transplant (349). These results suggest that 20-HETE is protective in renal transplant or at least may serve as an early clinical biomarker of transplant allograft function.

6.3. 20-HETE and cardiac ischemia reperfusion injury

Recent studies have indicated that 20-HETE may contribute to myocardial injury following IR. Nithipatikom *et al.* reported that CYP4A and CYP4F enzymes are expressed in the heart (334, 350). The synthesis and release of 20-HETE from dog heart is increased following IR injury. Inhibition of the synthesis of 20-HETE with HET0016 or blockade of the actions of 20-HETE with 5, 14 20-HEDE attenuated cardiac dysfunction and infarct size after IR (334, 350, 351). Moreover, inhibition of the formation of 20-HETE has a beneficial effect on the recovery of cardiac function following IR injury in diabetic rats (62). Conversely, exogenous administration of 20-HETE increased infarct size following IR in the heart (334, 350).

Ischemic preconditioning reduces infarct size following cardiac IR. The magnitude of the effect is similar to that seen following pretreatment of animals with an inhibitor of the synthesis of 20-HETE (300). Recent studies have indicated that ischemic preconditioning is associated with a reduction in the concentration of 20-HETE in coronary venous blood (352). Moreover, blockade of the synthesis of 20-HETE or administration of a 20-HETE antagonist potentiates the cardio-protective effects of ischemia preconditioning (352). These findings indicate that 20-HETE plays an important role in the pathogenesis of myocardial injury following IR. They also indicate that inhibitors of 20-HETE or receptor antagonists have therapeutic potential to protect against myocardial injury following IR.

Gross *et al.* (350) reported that the cardio-protective effects of 20-HETE inhibitors is associated with an increase in K^+ channel activity that is abolished by administration of the K_{ATP} channel blocker HMR-1098. While the mechanism by which 20-HETE contributes to myocardial injury remains to be fully determined, 20-HETE has been shown to increase the formation of ROS and myocardial dysfunction in isolated perfused rat heart following IR injury. The increase in oxidative stress associated with administration of 20-HETE was blocked by the NAPDH oxidase inhibitor apocynin. Moreover,

the cardioprotective action of HET0016 is associated with a reduction in oxidative stress following IR (131). These findings indicate that 20-HETE stimulates NADPH oxidase-derived ROS production, which aggravates IR-induced myocardial injury.

Bao *et al*. (353) reported that 20-HETE induces apoptosis of cultured cardiomyocytes by activation of mitochondria-dependent pathways. However, 20-HETE induced increase in inflammation as discussed earlier may also contribute to the adverse effects of 20-HETE on myocardial injury following IR (116, 173). Several studies have reported that sequence variants in the CYP4A11 gene that produces 20-HETE are linked with the incidence of myocardial infarction (MI) in human population studies (101, 104, 105). A haplotype based case control study that looked at three different SNPs in CYP4A11 gene (rs2269231, rs1126742, and rs9333025) found that the T-T-A haplotype was a protective genetic marker for MI in male Japanese (104).

There is also compelling evidence that 20-HETE may contribute to left ventricular hypertrophy, which is an important risk factor for heart failure, cardiac arrhythmias, myocardial infarction, and sudden cardiac death (44, 121, 153, 156, 354). In both patients and animal models, enzymes of the CYP4A and 4F families are upregulated in association with maladaptive cardiac hypertrophy associated with heart failure (96). For example, the expression of CYP4A11 mRNA is elevated in hypertrophic human hearts (169) and the expression of CYP4A3 and 4F4, and the production of 20-HETE was found to be elevated in isoproterenol-induced cardiac hypertrophy in rats (130, 355). Treatment of SD rats with fenofibrate was found to decrease CYP4A3 cardiac gene expression and protein levels and 20-HETE formation cardiac microsomes (356). Moreover, fenofibrate attenuated isoproterenol-induced cardiac hypertrophy, as determined by the increase in the expression ANP and BNP in the heart and the increase in the heart weight (356). Aryl hydrocarbon receptor (AhR) ligands, that promote cardiac hypertrophy, increased the expression of several CYP omega-hydroxylases, including CYP4F4, and increased 20-HETE production (357, 358). Inhibition of the synthesis of 20-HETE prevented AhR ligand-induced cardiac hypertrophy (358). In another study, 20-HETE levels in the heart were markedly elevated in ANG II-induced cardiac hypertrophy in rats (359). Recently, cardiac hypertrophy in this model was found to be inhibited by the antibiotic isoniazid, a well-known inducer of CYP2E1 which increases the formation of 19-HETE which serves as an endogenous competitive inhibitor of 20-HETE (25, 359). Overall, the results of these studies not only highlight the contribution of 20-HETE to cardiac hypertrophy, but the potential of using inhibitors of this pathway to prevent adverse cardiac remodeling.

The expression of CYP4A and 4F enzymes and 20-HETE production has recently been shown to be elevated in an animal model of heart failure (360). Moreover, the cancer chemotherapeutic agent doxorubicin (DOX), that produces cardiomyopathy, upregulates the expression of CYP4A1, CYP4A3, CYP4F1 and CYP4F4 in the heart, and enhances the formation of 20-HETE (361). Thus, DOX-induced cardiotoxicity is associated with early disturbances in the formation of 20-HETE that may contribute to the increase in oxidative stress. These cardiotoxic effects limit the usefulness of this important anticancer drug.

The signaling events linking 20-HETE to cardiac hypertrophy remain to be determined. Alsaad *et al*. suggested that 20-HETE may involve in activation of Rho kinase, ERK1/2, NF-KB, and NFAT pathways (96) in pathological cardiac hypertrophy as seen in other cell types. In adult rat cardiomyocytes, 20-HETE has been reported to activate PKC, which increases NADPH oxidase and ROS production, and in turn increases the activity of the L-type Ca^{2+} channel (362). Of note, PKC, NADH oxidase, ROS, and increased intracellular Ca^{2+} have been linked to pathological cardiac hypertrophy (363-365). It remains to be determined whether 20-HETE activates PKC via a receptor or directly interacts with PKC similar to the action of DAG (366). More recently, 20-HETE-mediated mitochondrial ROS production was implicated in ANG II-induced apoptosis of neonatal rat cardiac myocytes (367). Enhanced apoptosis of cardiac myocytes by 20-HETE (353) may have significance for the etiology of heart failure, which is associated with increased apoptosis of cardiac myocytes, as well as other forms of cell death.

7. SUMMARY

There is growing evidence that CYP450 of the 4A and 4F pathways and 20-HETE play an important role in a wide array of vascular dysfunction. The production of 20-HETE is altered in a variety of diseases. It acts as vasoconstrictor in vascular smooth muscle and promotes the myogenic response and autoregulation of renal and cerebral blood flow. 20-HETE also serves as a second messenger in the regulation of endothelial dysfunction, inflammation, angiogenesis, restenosis and IR injury in the brain, heart and kidney. These available data clearly indicate that 20-HETE is a potential novel pharmacological target and biomarker in a variety of diseases associated with vascular dysfunction and hypertrophy, and in ischemia-reperfusion injury in the brain, kidney and heart.

8. ACKNOWLEDGEMENTS

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Abbreviations: 17-ODYA: 17-octadecynoic acid; 20-COOH-HETE: 20-carboxy-eicosatatraenoic acid; 6, 15, 20-HEDE: 20-hydroxyeicosa-6(Z), 15(Z)-dienoic acid; 6, 15−20-HEDGE: N-(20-hydroxyeicosa-6(Z), 15(Z)-dienoyl) glycine; 20-SOLA: 2,5,8,11,14,17- hexaoxanonadecan-19-yl-20-hydroxyeicosa 6(Z), 15(Z)-dienoate; AA: arachidonic acid; ACI: acute cerebral ischemia; ACH: acetylcholine; Af-art: afferent arteriole; AKI: acute kidney injury; ANG II: angiotensin II; BI: balloon-injured; BK: the large conductance calcium sensitive potassium channel; BN: Brown Norway; CBF: cerebral blood flow; CKD: chronic kidney disease; CO: carbon monoxide; CSD: cortical spreading depolarization; CSF: cerebrospinal fluid; CSI: cortical spreading ischemia, COX: cyclooxygenase; COX2: cyclooxygenase 2; CXCR4: chemokine Receptor Type 4; CYP: cytochrome P450; Dahl SS: Dahl salt-sensitive; DAG: diacylglycerol;

DCI: delayed vasospasm and cerebral ischemia; DDMS: dibromo-dodecenyl-methylsulfimide; DiHETE: dihydroxyeicosatetraenoic acid; DOX: doxorubicin, ECM: extracellular matrix; EETs: epoxyeicosatrienoic acids; EGF: epidermal growth factor; ERK1/2: extracellular signal-regulated kinases ½; ET: endothelin; FGF_2: fibroblast growth factor 2; Hb: hemoglobin; HETE: hydroxyeicosatetraeonic acid; HET0016: N-hydroxy-N'-(4-butyl-2 methylphenyl)formamidine; HIF-1 alpha: hypoxia-inducible factor-1 alpha; HUVEC: human umbilical vein endothelial cell; IFN-gamma: interferon gamma; IL-1: interleukin 1; IR: ischemia reperfusion; LOX: lipoxygenase; MAPK: mitogen-activated protein kinases; MCA: middle cerebral artery; MCAo: occlusion of middle cerebral artery; MCP-1: monocyte chemoattractant protein-1; MMPs: metalloproteinases; NADPH: nicotinamide adenine dinucleotide phosphate; NF-KB: nuclear factor-kappa beta; NO: nitric oxide; NSAID: nonsteroidal anti-inflammatory drugs; PDGF: platelet-derived growth factor; PG: prostaglandins; PGI_2: prostacyclin; PKC: protein kinase C; PKD: polycystic kidney disease; PLA_2: phospholipase A_2; PPAR: peroxisome proliferator-activated receptors; RBF: renal blood flow; RAS: renin-angiotensin system; ROS: reactive oxygen species; SAH: subarachnoid hemorrhage; SBP: systolic blood pressure; SDF-1alpha: stromal cell-derived factor-1alpha; SHR: spontaneously hypertensive rat; TALH: thick ascending loop of henle; TGF: tubuloglomerular feedback; TNF alpha: tumor necrosis factor alpha; TRPC6: the transient receptor potential canonical 6 channel; TRPV1: the transient receptor potential vanilloid 1 channel; TS-011: N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide; TXA_2: thromboxane A_2; UGT: uridine 5'-diohosphoglucuronosyltransferase; VEGF: vascular endothelial growth factor; VSMC: vascular smooth muscle cells; vWF: von Willebrand factor; WIT003: 20-hydroxyeicosa-5(Z),14(Z)-dienoic acid

Key Words: 20-HETE, Cytochrome P450, Vascular Smooth Muscle, Endothelial Dysfunction, Vascular Remodeling, Vascular Inflammation, Ischemia Reperfusion, Stroke, Review

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