

20-hydroxyeicosatetraenoic acid, endothelial dysfunction and hypertension

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ABSTRACT

20-hydroxyeicosatrienoic acid (20-HETE) is a metabolite of arachidonic acid formed by the enzymatic activity of cytochrome P450 omega hydroxylases of the 4A and 4F isoform families (CYP 450 4A and 4F). The role of the metabolites of arachidonic acid (AA) formed by cyclooxygenases and lipoxygenases in mediating cardiovascular function have been studied and known for a long time. More recently, particularly in the last 10-15 years, the importance of the role that the CYP450 omega hydroxylase/20-HETE system plays in cardiovascular, inflammatory and neoplastic diseases, has captured the attention of the scientific community. In this brief review we discuss some of the more recent findings related to the function of 20-HETE in the cardiovascular system. In particular we focus on the interactions of 20-HETE with the intracellular pathways of nitric oxide and the renin angiotensin system and discuss how it plays a role in oxidative stress, the development of endothelial dysfunction and experimental and human hypertension. To date the research strongly suggests that 20-HETE is an important and central mediator of cardiovascular function, and that alterations in the normal regulation of the CYP450/20-HETE system play a role in the pathogenesis of many disorders. There is great potential for the development of therapeutic agents to modify the activation and activity of this system in order to prevent and/or treat hypertension.

Key words: 20-HETE, hypertension, endothelial dysfunction

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Original submission:

18 April 2011;

Revised submission:

13 June 2011;

Accepted:

13 June 2011.

Med Glas Ljek komore Zenicko-dobojskog kantona 2011; 8(2):170-180

INTRODUCTION

Cardiovascular disease is a major health problem worldwide. The World Health Organization estimated the age standardized death rate (per 100,000) for cardiovascular diseases (ischemic heart, and cerebrovascular disease combined) as 318.5 in Croatia and 178.8 in the United States (1). Hypertension, a significant contributor of risk to the development and exacerbation of cardiovascular diseases, is highly prevalent in both the US and Croatia. In 2005-2008, 30.9% of all U.S. adults 18 years and older were hypertensive. An additional 28% of U.S. adults had prehypertension (systolic BP 120-139 mm Hg or diastolic BP 80-89 mm Hg, and not pharmacologically treated for high BP) (1, 2). The prevalence of uncontrolled hypertension in Croatia, among those 18 years and older, is even higher at 40.5% in men and 34.9% in women (3).

A common finding in all types of hypertension (as well as many diseases associated with hypertension, i.e. diabetes, metabolic syndrome) is endothelial dysfunction, characterized by an imbalance in the expression of and sensitivity to vasodilator and vasoconstrictor agents resulting in increased vascular tone and thus an increase in resistance to flow (4, 5). Many vasoactive factors involved in endothelial pathology have been studied extensively in this regard, including nitric oxide (NO), endothelin, reactive oxygen species (ROS), renin-angiotensin-aldosterone (RAAS), endothelial derived hyperpolarizing factor (EDHF), and enzymatic end products of cyclooxygenases, lipoxygenases.

Of more recent research interest, particularly within the last 10 years, are the products of the catalytic activity of cytochrome P450s monooxygenases on the intramembrane fatty acid arachidonic acid (AA). An increase in the concentration of intracellular Ca^{2+} activates calcium sensitive phospholipase A_2 which in turn stimulates the release of arachidonic acid (AA) from membrane phospholipids. Free AA is then metabolized by ω -hydroxylases and epoxigenases into hydroxyeicosatrienoic acids (HETEs) or epoxyeicosatrienoic acids (EETs), respectively (6). The research suggests that these enzyme systems play an important role in the regulation of renal sodium reabsorption, inflammation, ischemia and reperfusion injury, and peripheral vascular tone. 20-HETE has

antihypertensive actions, regulation sodium and water retention and excretion in the renal tubules and prohypertensive actions in the vasculature. We will focus this brief review on what we know to date about 20-hydroxyeicosatrienoic acid (20-HETE), a catalytic product of CYP 450 4A (rat) and 4F (rat and human) ω -hydroxylase isoforms from AA, and its involvement in the regulation of microcirculatory vascular tone and blood flow, interaction with other vasoactive agents and how it might play a role in endothelial dysfunction and in experimental and human hypertension.

20-HYDROXYEICOSATRIENOIC ACID (20-HETE)-MECHANISMS OF ACTION AND TISSUE EXPRESSION

20-HETE is a potent vasoconstrictor of arterioles in renal (7-12), mesenteric (13), skeletal muscle (14, 15) and cerebral (16, 17) microcirculatory beds. 20-HETE causes vasoconstriction by inhibition of large conductance calcium-activated potassium channels (BK_{Ca}) via activation of protein kinase C (PKC) (18), depolarizing the membrane and increasing $[Ca^{2+}]_i$ (8, 9, 17). There is also evidence that 20-HETE increases L-type Ca^{2+} current (19, 20). 20-HETE stimulates the production of vasoactive substances, increases the sensitivity of vascular smooth muscle (VSM) cells to them, and acts as a second messenger for many of these agents (21-37). In addition, it has been demonstrated that 20-HETE activates Rho-kinase and phosphorylation of myosin light chain 20 (MLC20) and sensitizes the contractile apparatus to Ca^{2+} (38, 39).

CYP450 monooxygenases have been measured in hepatic and extrahepatic tissues of many species, either measured directly by Western Blot (protein expression) and/or RT-PCR (expression of mRNA of specific isoforms) or indirectly by measures of their catalytic product, 20-HETE, by high performance liquid chromatography (HPLC) or gas chromatography/mass spectrometry (GS/MS). CYP4A isoforms have been detected in rats (11, 14-16, 40-72), mice (73-75), rabbits (9, 76-84), cats (17, 19), dogs (85, 86) and humans (87-94).

20-HETE AND NITRIC OXIDE (NO)

Nitric oxide, an endogenously produced free radical, plays an important role in vascular homeostasis. Disorders in the enzyme systems that cre-

ate NO are a common factor in human and many experimental forms of hypertension and play an important role in endothelial dysfunction⁹⁵⁻⁹⁷. NO is synthesized in endothelial cells from L-arginine through the action of two constitutively expressed nitric oxide synthases; endothelial derived NO synthase (eNos, NOX3) and neuronal NOS (nNOS, NOS1) and one inducible isoform, iNOS or NOS2. The biological functions of NO include vasodilation, antiinflammation, antithrombosis and facilitation of renal sodium and water excretion. The regulation of NO synthesis is very complex and beyond the scope of this review. However, it has been generally thought that the actions of NO were primarily mediated through cyclic guanosine monophosphate (cGMP) secondary to stimulation of soluble guanylyl cyclase (sGC). It is now known, however, that there are sGC independent pathways for the regulation of NO since inhibitors of sGC, at least in some vascular beds, do not completely prevent NO induced vasorelaxation. One of those important regulators appears to be 20-HETE (98). Several studies demonstrate that 20-HETE plays an important role in the regulation of NO activity and conversely that NO plays an important role in the regulation of 20-HETE synthesis in several microcirculatory beds. Inhibition of the synthesis of 20-HETE by a selective, mechanism-based inhibitor of P450 enzymes of the 4A family, DDMS (dibromo-dodecanyl-methylsulfimide), or 17-ODYA (17-octadecynoic acid) a suicide substrate inhibitor, attenuated the vasodilation of isolated renal interlobular arterioles to the NO donor, sodium nitroprusside (SNP) (59, 62). The authors suggest that what this demonstrated is that a large percentage of NO induced vasodilation is cGMP independent and occurs through inhibition of the production of 20-HETE and the subsequent activation of BK_{Ca} channels and hyperpolarization of the membrane. Similarly, in middle cerebral arteries inhibition of 20-HETE synthesis by DDMS attenuated vasodilation to SNP (66) and DEA-NONOate (99). This cGMP independent effect seems to be primarily important in the smaller cerebral vessels, i.e. MCAs and not in the larger basilar (66) or pial arteries (100). Oyekan et al., found that competitive inhibition of NOS with N^o-nitro-L-arginine methyl ester (L-NAME) revealed a major vasoconstriction in renal arteries that was mediated by a metabolite of CYP 450 monooxygenases (101). This metabolite was most

likely 20-HETE, since this L-NAME induced increase in renal vascular resistance was blocked with a selective inhibitor of ω -hydroxylases. In another study by these investigators, the NO donor sodium nitroprusside inhibited the formation of 20-HETE in a dose dependent manner from renal microsomes (68), whereas an inhibitor of cGMP, the second messenger of NO, had no effect on the production of 20-HETE. Inducible Nos is known to be a mediator of the systemic effects of septic shock since inhibition of iNOS activity attenuates the endotoxin induced vasodilation, hyporeactivity and drop in blood pressure (102-105). It has been demonstrated that this effect is not due only to the direct effects of inhibition of iNOS but also due to the disinhibition and increased activity of vasoconstrictors including, 20-HETE (102-105). Administration of L-arginine, the substrate for NO synthases reduced the production of 20-HETE in renal microsomes treated with cyclosporine A an immunosuppressant that is known to increase the production of 20-HETE and at least partially mediates the toxic effects of this agent (106). The ability of NO to inhibit the renal toxic effects of cyclosporin A In rats with an increased production of 20-HETE, due to intravenous injection of adenoviral vectors carrying CYP4A2 (Adv-CYP4A2), the NO-dependent component of acetylcholine-induced vasorelaxation in renal interlobar arteries was impaired (107). Cheng, et al. studied the ability of 20-HETE to regulate NO and demonstrated that 20-HETE decreases NO bioavailability by eNOS uncoupling (108) at least partially through blocking the association of eNOS with heat shock protein 90 (HSP90). In another study by this same group it was found that 20-HETE inhibits NO production, stimulates I κ B phosphorylation, a key step in the activation of the NF- κ B pathway, and attenuates acetylcholine induced vasodilation by a mechanism that involves the activation of the tyrosine kinase/EGFR and MAPK pathways (26). Wang, et al. found that inhibition of NO with L-NAME increased the production of 20-HETE by 3x in microvessels of rats in late pregnancy (109).

20-HETE AND THE RENIN-ANGIOTENSIN-SYSTEM (RAS)

The cardiovascular effects of angiotensin II (AII), known to be a critical player in the development of endothelial dysfunction (95, 110), are media-

ted through two known receptors AT1 and AT2. Typically the AT1 receptor is thought to mediate vasoconstriction and sodium retention and AT2 to mediate vasodilation and sodium excretion (110). The second messenger intracellular signaling pathways of angiotensin are complex and beyond the focus of this review (110), however, since AII activates phospholipases and increases arachidonic acid it is reasonable to assume that AII would also increase the synthesis of 20-HETE. The research bears this out. Infusion of 50ng of AII into the rabbit isolated perfused kidney increased the production of 20-HETE by 7x (78). Similarly, incubation of preglomerular microvessels with AII doubled the production of 20-HETE (111). Angiotensin II induced constriction of the isolated rabbit afferent arteriole was blunted with inhibition of 20-HETE synthesis by 17-ODYA (112). An interesting observation in that study was that miconazole, an inhibitor of the production of epoxyeicosatrienoic acids (EETs) augmented the AII induced constriction (112). The authors postulate that the latter occurred through the AT2 receptor since the application of a specific blocker of that receptor, PD 123319, also augmented the AII induced constriction. A unique finding has been reported in rabbit smooth muscle cells by Muthalif, et al. in which 20-HETE, created enzymatically from arachidonic acid freed from the plasma membrane by the actions of AII on calcium/calmodulin dependent kinase (CaMKII)/phospholipase A₂, in a positive feedback loop in turn activated the Ras/mitogen-activated protein kinase (MAPK) that in turn increased the activation of PLA₂ (80). This same group also demonstrated that 20-HETE serves as a second messenger/mediator of the AII stimulation of phospholipase D via the ras/extracellular signal-regulating kinase (ERK) pathway (113). AII induced vasoconstriction of the rat mesenteric superior artery (114) and in cremasteric arterioles of both normotensive and reduced renal mass hypertensive Sprague Dawley rats (115) was significantly attenuated by a selective inhibitor of 20-HETE synthesis, DDMS. In a mouse knock out model of increased synthesis of 20-HETE (74), Cyp4a14 null (KO) mice, systolic blood pressure was elevated and renal vascular responses to angiotensin II enhanced (116). In a rat model of diabetes, a disease which is associated with endothelial dysfunction and hypertension, Yousif, et al. described an enhanced constriction of the isolated

perfused mesenteric artery and the renal artery to AII which is attenuated with specific inhibition of 20-HETE synthesis by N-hydroxy-N'-(4-n-butyl-2-methylphenyl) Formamidine (specific inhibitor of 20-HETE synthesis, HET-0016) (117). They suggest that the 20-HETE- induced increased sensitivity to AII is possibly mediated through upregulated expression or activity of the CaMKII or Ras-GTPase pathways (117).

That 20-HETE plays an important role in angiotensin II related hypertension, was demonstrated by Alonso-Galicia, et al. since inhibition of 20-HETE with DDMS reduced mean arterial pressure that had been increased by acute or chronic infusions of AII in Sprague Dawley rats (118). Similarly, in a model of AII dependent hypertension, the Ren-2 transgenic rat, inhibitors of 20-HETE prevented the increase in blood pressure and the concomitant renal damage (119). In a very interesting study by Sodhi, et al. they transduced a lentivirus expressing the CYP4A2 cDNA into Sprague Dawley rats which caused an increase in CYP4A expression and 20-HETE synthesis (22). These changes were accompanied by an increase in systolic blood pressure, an increase in plasma levels of AII, and increased tissue expression of AT1 receptor and angiotensin converting enzyme (ACE). All of these were attenuated by treatment with lisinopril (ACE inhibitor), losartan (AT1 receptor blocker), HET0016 or 20-hydroxyeicosa-6(Z), 15(Z)-dienoic acid (20-HETE antagonist, 20-HEDE). The investigators conclude that this is a model of 20-HETE induced hypertension that is mediated through an increase in activation of ACE. This association between 20-HETE and AII has also been reported in humans with renal vascular disease (RVD) and hypertension. In a cross sectional study, urinary excretion of 20-HETE was reduced, and plasma levels of 20-HETE were increased and correlated positively with plasma renin activity in subjects with RVD (120).

20-HETE AND OXIDATIVE STRESS

Oxidative stress is an imbalance between the production of reactive oxygen species (ROS) that are normally produced by cellular processes and the ability of cells to convert them into water. It is considered a chief cause of endothelial dysfunction and has been found to play an important role in the development of cardiovascular

disease including hypertension (121). An excess of ROS often arise from increased expression and/or activity of NADPH oxidases and xanthine oxidase which produce a number of ROS including superoxide ($\bullet\text{O}_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($\bullet\text{OH}$) and peroxynitrite (ONOO^-). Normally, ROS are reduced by superoxide dismutases (SODs) but these systems can become overwhelmed. Many endogenous and exogenous factors contribute to the development of ROS, including NO and AII. Given the findings summarized above it should not be surprising that 20-HETE contributes to the creation of ROS as well. Cheng, et al. found that in bovine aortic endothelial cells (BAEC) 20-HETE inhibited the production of NO by eNOS uncoupling, that is by blocking the interaction of eNOS with the 90-kDA heat shock protein (HSP90) and that this was associated with a 25% increase in superoxide production (108). Furthermore the addition of L-NAME or 19 (R) HETE, a competitive inhibitor of 20-HETE, completely inhibited the production of superoxide in the BAECs. This effect of 20-HETE on eNOS uncoupling was confirmed in murine aortic rings (122). These investigators suggest that this effect of 20-HETE was mediated by AMP-activated protein kinase (AMPK) since chronic activation of AMPK by AICAR reduced endothelial dysfunction in the rings. In rats with increased 20-HETE synthesis induced by intravenous injections of adenoviral vectors carrying the CYP4A2 construct, renal interlobar arteries produced increased superoxide ions and the expression of gp91phox, a major component of the NADPH oxidase, was elevated in aortic endothelial cells (107). Singh, et al. demonstrated that 20-HETE played a role in the development of hypertension induced by administration of the androgen, 5 α -dihydrotestosterone (DHT) (123). In the treated rats, 20-HETE synthesis was increased in renal interlobar arteries and treatment with HET0016 returned blood pressure to normal. The critical finding in terms of our discussion here is that the DHT treatment increased the expression levels of p47^{phox} and gp91^{phox}, components of the vascular superoxide-generating reduced nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase system. HET0016 markedly inhibited DHT-induced p47^{phox} and gp91^{phox} protein levels. Isolated middle cerebral arteries from a genetic model of spontaneous hypertension,

stroke, and endothelial dysfunction, the spontaneously hypertensive stroke prone rat (SHRSP), produced almost twice as much superoxide as the WKY controls. This elevation in $\bullet\text{O}_2^-$ was significantly reduced with HET0016 in the vessel bath. Furthermore, chronic treatment with HET0016 restored endothelium dependent vasodilation in this model (124). Recently, Zeng et al. found that the 20-HETE induced increase in L-type calcium channel current in cardiomyocytes was mediated through an increase in protein kinase C (PKC) which in turn phosphorylates the NADPH oxidase and increases the production of superoxide. The authors suggest that the upregulation of this mechanism may play an important role in the injuries due to myocardial ischemia.

20-HETE IN HUMAN HYPERTENSION

To date the relationship among 20-HETE, endothelial dysfunction and hypertension has not been studied in humans to the same extent as in laboratory animals. However, it is known that the human kidney cortex metabolizes arachidonic acid to 20-HETE via the enzymatic activity of CYP4F2 and CYP4A11 (92, 93) and that 20-HETE is excreted in urine (88, 94, 125-128) confirming that 20-HETE is indeed synthesized *in vivo* in the human. 20-HETE has also been measured in human platelets and neutrophils (129). Ward, et al. studied the relationship between levels of 20-HETE urinary excretion and endothelial dysfunction (measured by flow mediated dilation (FMD)) in normotensive and hypertensive men and women (127). They found that in subjects with a very low % of FMD in the brachial artery that 20 urinary excretion was significantly higher than in those with a higher % of FMD. Interestingly, in women who were postmenopausal in this study, but not men there was a positive correlation with blood pressure and 20-HETE excretion. In addition, hypertensive women had a higher 20-HETE excretion than normotensive women (127). In another study of hypertensive subjects, these same investigators found a positive correlation between 20-HETE urinary excretion and measures of oxidative stress (i.e. F₂-isoprostanes, gamma-Glutamyl transpeptidase) (128). Yang, et al. found a positive correlation between plasma levels of 20-HETE, 24 hr systolic blood pressure, and F₂-isoprostanes and a negative correlation between plasma nitrites and

24 hr systolic blood pressure in overweight and obese men and women (130).

There have been several studies that have found correlations between functional variants of human CYP 4F isoforms, the production of 20-HETE and the presence of cardiovascular disease (131-138). Some of the variants were associated with an increased production of 20-HETE while others with a decreased production of 20-HETE which probably reflects the fact that 20-HETE has antihypertensive actions at the level of the kidney tubules but prohypertensive actions at the level of blood vessels. Stec, et al. expressed proteins from recombinant baculoviruses from four different human CYP4F2 variants in Sf9 insect cells. The presence of the M433 allele, W12/M433, or G12/M433 decreased 20-HETE production to 56-66% of control (133). Ward, et al. reported a significant association of the single nucleotide polymorphism *CYP4F2* GA/AA genotype with elevated systolic BP and significantly increased urinary 20-HETE excretion in normotensive and hypertensive subjects. On the other hand a SNP

CYP4A11 TC/CC was significantly associated with decreases in 20-HETE excretion (137).

In conclusion, taken together the findings from these studies point to a significant contribution of the CYP monooxygenase/20-HETE system to the endothelial and vascular dysfunction that is widely found in cardiovascular diseases, in particular hypertension. In addition to the results of studies already cited, 20-HETE mediates and/or augments the actions of other vasoconstrictors, such as, endothelin (24, 129, 139) and serotonin (140). 20-HETE is also thought to play a significant role in the inflammatory process (103, 141, 142), the regulation of myogenic tone (143-145) and cancer (146, 147). Clearly the research evidence strongly suggests that 20-HETE plays a prominent role in cardiovascular and immunological systems. Further research on the development of therapeutics that will modify the actions of this system is warranted.

ACKNOWLEDGMENT/DISCLOSURES

Competing interests: none declared.

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