Prostaglandins & other Lipid Mediators xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Review

# Vascular actions of 20-HETE

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ARTICLE INFO

*Article history:*
Available online xxx

*Keywords:*
20-HETE
Renin-angiotensin system
Vascular remodeling
Vascular dysfunction
Vascular inflammation
Angiogenesis

ABSTRACT

20-hydroxyeicosatetraenoic acid (20-HETE) is a metabolite of arachidonic acid that exhibits a myriad of biological effects in the vascular system. This review discusses the current knowledge related to the effects of 20-HETE on vascular reactivity, activation, and remodeling, as well as its role in vascular inflammation and angiogenesis. The information explaining how 20-HETE and the renin-angiotensin system interact to promote hypertension, vasoconstriction, and vascular dysfunction is summarized in this article. 20-HETE enhances vascular inflammation and injury in models of diabetes, ischemia/reperfusion, and cerebrovascular oxidative stress. Recent studies also established a role for 20-HETE in normal and pathological angiogenesis conditions. This review will also discuss the molecular mechanisms through which 20-HETE induces these vascular actions. Potential additional studies are suggested to address shortcomings in the current knowledge of 20-HETE in the vascular system.

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**Contents**



## 1. Introduction

20-hydroxyeicosatetraenoic acid (20-HETE) is an eicosanoid that regulates a myriad of actions in the vascular system. It is synthesized through metabolism of arachidonic acid (AA) by cytochrome P450 (CYP) ω-hydroxylases. Several isoforms of CYP ω-hydroxylases, which are the main producers of 20-HETE, are expressed in humans, mice, and rats. A number of research groups have established a role for 20-HETE in the vascular system through the use of cell, animal, and human models.

The main focus of this review is to discuss the effects of 20-HETE in the vascular system and the mechanisms involved in these processes. 20-HETE has an integral interaction with the renin-angiotensin system leading to a feed-forward mechanism that perpetuates vascular dysfunction and hypertension. The mechanisms involving 20-HETE in vascular reactivity, activation, and remodeling have been extensively studied. Changes in 20-HETE production also regulate vascular inflammation in diabetes, ischemia/reperfusion and cerebrovascular oxidative stress injury. Several studies established that 20-HETE enhances angiogenesis in

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http://dx.doi.org/10.1016/j.prostaglandins.2015.03.002
1098-8823/Published by Elsevier Inc.

**Table 1**
Summary of CYP450 ω-hydroxylases that produce 20-HETE in humans, rats, and mice.

| Species | Cytochrome P450 ω-hydroxylases responsible for 20-HETE production |
|---|---|
| Human | *CYP4A11* (CYP4A11); *CYP4A22* (CYP4A22); *CYP4F2* (CYP4F2); *CYP4F3* (CYP4F3) |
| Rat | *Cyp4a1* (CYP4A1); *Cyp4a2* (CYP4A2); *Cyp4a3* (CYP4A3); *Cyp4a8* (CYP4A8) |
| Mouse | *Cyp4a10* (CYP4A10); *Cyp4a12* (CYP4A12) |

*gene*; (protein).

normal and pathological conditions. Here we summarize the literature related to these vascular actions of 20-HETE and what is known regarding the mechanisms through which 20-HETE regulates these processes. We also address currently unanswered questions that are of interest to further advance the understanding of the vascular actions of 20-HETE.

## 2. 20-HETE biosynthesis

20-HETE is derived from metabolism of AA by CYP ω-hydroxylases of the CYP4A and CYP4F subfamilies. Arachidonic acid is a polyunsaturated fatty acid that is a major component of membrane phospholipids. AA is liberated from the plasma membrane by phospholipase A2. To produce 20-HETE, the CYP ω-hydroxylases insert a hydroxyl group at the terminal $sp^3$ carbon group of AA [1]. There are several isoforms of CYP4A/CYP4F responsible for the production of 20-HETE, which are summarized in Table 1. In humans, these isoforms are CYP4A11, CYP4A22, CYP4F2, and CYP4F3 [2–4]. The predominant 20-HETE synthase in humans is CYP4F2 followed by CYP4A11. CYP4F2 exhibits high activity in leukocytes and kidneys [4,5]. In mice, the 20-HETE producing enzymes include CYP4A10 and CYP4A12 [6]; CYP4A12 is the primary 20-HETE synthase [6,7]. In rats the 20-HETE producing enzymes include CYP4A1, CYP4A2, CYP4A3, and CYP4A8 [8–10].

Various studies have revealed functional variants in both human *CYP4F2* and *CYP4A11*. Population differences have been observed in the *CYP4A11* loss-of-function variant 8590T>C with higher frequency being observed in African-American and some Japanese populations [11]. *In vitro* experiments have demonstrated that several human *CYP4F2* variants result in reduced production of 20-HETE [12]. In contrast to these *in vitro* results, an *in vivo* study revealed that the *CYP4F2* V433M polymorphism was associated with increased urinary excretion of 20-HETE [13]. These discrepancies could be due to different factors regulating 20-HETE production in humans as compared to isolated *in vitro* systems. Caution should be taken when comparing *in vitro* results to human populations.

Vascular synthesis and release of 20-HETE occurs primarily in vascular smooth muscle cells [14–20]. These cells are not the sole source of 20-HETE; it can arise from myeloid cells in the peripheral blood and bone marrow [21–23]. 20-HETE is also produced in human neutrophils and platelets [24]. Neutrophil and platelet 20-HETE production is increased by Ang II and endothelin-1 treatment [24]. Androgen is also a potent inducer of 20-HETE synthesis [25]. Interestingly, endothelial progenitor cells, which are involved in postnatal neovascularization, produce 20-HETE [26]. In contrast, vascular endothelial cells in most circulatory beds are devoid of 20-HETE synthase activity [27].

## 3. 20-HETE and the renin-angiotensin system (RAS)

The renin-angiotensin system (RAS) serves a critical role in the regulation of blood pressure. The RAS is comprised of several components including renin, angiotensin-converting enzyme (ACE), and angiotensin II type 1 receptor (AT1R). Formation of the vasoactive octapeptide angiotensin II (Ang II) occurs through stepwise degradation of angiotensinogen. Angiotensinogen, which is primarily produced by the liver, is first converted to the decapeptide angiotensin I (Ang I) *via* the enzyme renin. Ang I is further cleaved by ACE to its vasoactive Ang II form. The vasomotor actions of Ang II are primarily *via* activation of the AT1R within the vasculature resulting in vasoconstriction and a variety of other vascular, renal, and fluid balance effects [28,29].

Several studies document the complex interactions between the RAS and 20-HETE in hypertension. The release and synthesis of 20-HETE is induced by several autacoids including endothelin-1 [30–32] and Ang II [33,34]. Ang II stimulates the synthesis and release of 20-HETE from isolated rat preglomerular microvessels to enhance the pressor effects of Ang II [35–37]. 20-HETE mediates the mitogenic [15,33,38–40] and vasoconstrictor effects of Ang II by mediating hypertrophy and hypertension through activation of the Ras/MAP kinase pathway [41]. Thus, inhibition of 20-HETE synthesis attenuates the renal pressure response to Ang II as well as inhibits the development of Ang II-dependent hypertension [42,43]. Interestingly, Ang II's actions on vascular cells parallel the biological actions of 20-HETE: stimulation of superoxide/ROS, NF-kB activation, and induction of inflammatory adhesion molecules (ICAM/VCAM) [44–52]. Conversely, recent studies identified 20-HETE as a potent inducer and transcriptional activator of endothelial ACE expression in microvascular endothelial cells [53,54].

Animal models of hypertension that demonstrate increased vascular 20-HETE production are also RAS-mediated and–dependent. These models include the spontaneous hypertensive rat (SHR) [55,56] and androgen-induced hypertension [17,57,58]. Androgen influences renal 20-HETE synthesis in spontaneously hypertensive rats [59]. Sprague-Dawley rats overexpressing CYP4A2 in the vascular endothelium exhibit increased 20-HETE production and hypertension [6,17,60]. The increase in blood pressure coincides with increased expression of vascular ACE and is normalized by ACE inhibition or AT1R blockade [53,60]. These observations suggest a feed forward mechanism by which the 20-HETE axis and the RAS work in concert to promote vascular dysfunction and hypertension [61]. The interaction between the RAS and 20-HETE is depicted in Fig. 1.

## 4. Vascular reactivity, endothelial dysfunction and endothelial activation in response to 20-HETE

20-HETE sensitizes vascular smooth muscle cells to a variety of constrictor stimuli, including Ang II, phenylephrine and endothelin [18,62,63] through several mechanisms. 20-HETE sensitizes smooth muscle through inhibition of the large conductance $Ca^{2+}$-activated $K^+$ (BKCa) channels. Inhibition of BKCa channels depolarizes plasma membranes, increases $Ca^{2+}$ entry through L-type $Ca^{2+}$ channels, and elevates cytosolic [$Ca^{2+}$] to potentiate vasoconstriction [14,15,64]. 20-HETE can also increase conductance of the L-type $Ca^{2+}$ channels through PKC activation. In small coronary arteries, 20-HETE activates Rho kinase resulting in phosphorylation of myosin light chain to increase sensitivity of the vessel to $Ca^{2+}$ [41]. 20-HETE not only induces vasoconstriction but also reduces vascular relaxation. For example, 20-HETE attenuates the relaxing response to acetylcholine in renal interlobar arteries pre-constricted with phenylephrine [65–67]. These vascular effects depend on the complex relationship between RAS and 20-HETE as concurrent pretreatment of vessels with 20-HETE and an ACE inhibitor (Lisinopril) or AT1R blocker (Losartan) attenuate 20-HETE's effects [54].

Nitric oxide (NO) produced by the vascular endothelium is an important mediator in the defense against vascular injury and

Please cite this article in press as: Hoopes SL, et al. Vascular actions of 20-HETE. Prostaglandins Other Lipid Mediat (2015), http://dx.doi.org/10.1016/j.prostaglandins.2015.03.002