Published in final edited form as:

Curr Top Med Chem. 2013 ; 13(12): 1429–1440.

CYP4 Enzymes as potential drug targets: focus on enzyme multiplicity, inducers and inhibitors, and therapeutic modulation of 20-hydroxyeicosatetraenoic acid (20-HETE) synthase and fatty acid ω -hydroxylase activities

Katheryne Z. Edson and

Department of Medicinal Chemistry, University of Washington, Box 357610, Seattle, WA 98195

Allan E. Rettie

Department of Medicinal Chemistry, University of Washington, Box 357610, Seattle, WA 98195,
Phone: 206-685-0615, Fax: 206-685-3252

Allan E. Rettie: rettie@u.washington.edu

Abstract

The Cytochrome P450 4 (CYP4) family of enzymes in humans is comprised of thirteen isozymes that typically catalyze the ω -oxidation of endogenous fatty acids and eicosanoids. Several CYP4 enzymes can biosynthesize 20-hydroxyeicosatetraenoic acid or 20-HETE, an important signaling eicosanoid involved in regulation of vascular tone and kidney reabsorption. Additionally, accumulation of certain fatty acids is a hallmark of the rare genetic disorders, Refsum disease and X-ALD. Therefore, modulation of CYP4 enzyme activity, either by inhibition or induction, is a potential strategy for drug discovery. Here we review the substrate specificities, sites of expression, genetic regulation, and inhibition by exogenous chemicals of the human CYP4 enzymes, and discuss the targeting of CYP4 enzymes in the development of new treatments for hypertension, stroke, certain cancers and the fatty acid-linked orphan diseases.

Keywords

20-HETE; CYP4; HET0016; Hypertension; Stroke; Cancer; Refsum Disease; X-ALD

Introduction

The Cytochrome P450 4 (CYP4) family of enzymes typically metabolizes fatty acids, including many important cellular signaling eicosanoids. Eicosanoids are the oxidation products of 20-carbon essential fatty acids, such as arachidonic acid, that constitute a complex network of molecular controls throughout the human body. 20-Hydroxyeicosatetraenoic Acid (20-HETE), generated mainly by CYP4A and/or CYP4F enzymes, is a key arachidonic acid metabolite that exhibits a plethora of biological functions, such that pharmacological manipulation of tissue 20-HETE levels has promise for

the treatment of several disease states. The purpose of this article is, firstly, to discuss the biochemistry of CYP4 enzymes with an emphasis on their multiplicity, species orthologs, chemical induction and inhibition. Down- and up-regulation of salient human CYP4 enzyme activity is of particular importance in the context of CYP4 enzymes as drug targets, which is the focus of the second half of this review.

CYP4 Enzymes: Overview of catalysis, expression, regulation, and inhibition

CYP4 enzymes are heme-containing monooxygenases with an unusual ability to catalyze hydroxylation of a terminal carbon atom on an unactivated alkyl chain. This is in contrast to most other human P450 enzymes, which also catalyze aliphatic chain oxidations, but favor internal hydroxylations (ω -1, ω -2, ω -3, etc.) due, in part, to the lower energetic cost of breaking a substituted C-H bond as opposed to a terminal C-H bond. The topology of the CYP4 active site and steric constraints imposed by the nearby amino acids are important factors dictating product regioselectivity. A likely additional important structural consideration is the unique covalent link to the heme cofactor that is present in numerous members of the CYP4 family of enzymes [1, 2]. This unusual covalent ester bond formed between a glutamate residue on the CYP4 I-helix and a methyl substituent on the B-type heme may rigidify the active site to enhance regio-control of substrate hydroxylation (Figure 1). It is important to note that this ω -regioselectivity is not absolute, and most CYP4 enzymes produce a mixture of end-chain hydroxylated products. The ratios of ω : ω -1, ω -2, and ω -3 hydroxylated products can range from >20:1 to 0:1 and can vary widely for each enzyme/substrate pair. Two of the twelve human CYP4 enzymes, CYP4F8 and CYP4F12, have a glycine residue in place of the glutamic acid on the I-helix and do not possess a covalently bound heme. Perhaps as a result, these enzymes catalyze the hydroxylation of eicosanoids such as prostaglandin H2 and arachidonic acid at the ω -2 and ω -3 positions, and are thus not strictly ω -hydroxylases [3, 4]. CYP4X1 and CYP4Z1 also lack a glutamic acid in the I-helix and likely have no covalently bound heme cofactor, however the substrate specificity and product regio-selectivity of these enzymes are not yet well defined.

The twelve human CYP4 isoforms are distributed among six CYP4 subfamilies whose constituent enzymes are shown in Figure 1. The CYP4A, B, X, and Z genes are clustered on chromosome 1, while the CYP4F and CYP4V genes reside on chromosome 19 and 4, respectively. P450 enzymes are grouped into families and subfamilies based on their amino acid sequence similarity. Enzymes with >40% amino acid identity are classified into the same family, and enzymes with >55% identity are members of the same subfamily. The substrate selectivity, human protein expression levels, and regulation of expression for each CYP4 subfamily are described below.

The CYP4A subfamily—Within the human CYP4A subfamily, CYP4A11 is very efficient at ω -hydroxylation of medium-chain length (C10-C16) fatty acid substrates. CYP4A22 is the only other known human CYP4A enzyme. However, CYP4A22 is expressed at very low levels in few tissues and may not be a functional enzyme [5]. CYP4A22 does not hydroxylate lauric acid, the prototypical CYP4A substrate, although it shares 95% sequence homology with CYP4A11. CYP4A11 catalyzes lauric acid hydroxylation with a V_{max} of $38 \pm 8 \text{ min}^{-1}$, K_m of $200 \pm 50 \mu\text{M}$, and product ω : ω -1 ratio

in excess of 10:1 [6]. As the chain length of the substrate increases beyond C12 this ratio decreases. For example, the ω : ω -1 ratio for arachidonic acid hydroxylation (producing 20- and 19-HETE) is only 4.7:1 [7]. CYP4A11 is expressed primarily in the liver and kidney. In human liver and renal cortex microsomes the specific contents of the enzyme have been reported to be as high as 48 ± 28 pmol/mg and 32 ± 18 pmol/mg ($n=9$), respectively, as determined by immunoquantitative western blotting [8].

Amongst CYP4 enzymes, those of the CYP4A subfamily are the most studied in regard to regulation of hepatic expression. Most of this work has been performed in the rat and mouse, experimental animals that possess multiple functionally important CYP4A enzymes (CYP4A1, 2, 3, and 8 in rats and CYP4a10, 12, and 14 in mice). It is well established that the nuclear receptor, PPAR α , mediates marked induction of mouse and rat CYP4A mRNA, protein, and lauric acid hydroxylase activity in response to the fibrate class of hypolipidemic drugs, as well as in states of fasting and diabetic ketoacidosis [9, 10]. Under these conditions, PPAR α is activated due to increased levels of free fatty acids resulting from adipocyte lipolysis and increased ketogenesis as the cells attempt to make energy from stored fatty acids to counteract glucose shortage. However, a number of studies have failed to demonstrate PPAR α -mediated induction of peroxisome proliferation-associated genes by fibrates in human hepatocytes and HepG2 cells, although the orthologous genes were substantially up-regulated in rat hepatocytes [11, 12]. This led to speculation that PPAR α does not mediate CYP4A11 induction in humans. However, human CYP4A11 transgenic mice exhibit a ~3-fold increase in CYP4A11 mRNA and protein expression in response to both fibrate agonists for PPAR α and fasting states, and CYP4A11 was no longer inducible in PPAR α deficient CYP4A11 transgenic mice [13]. Increased CYP4A11 mRNA and protein expression in response to fibrates and other more potent PPAR α agonists in human hepatocytes (1.5–4 fold) has since been shown, although induction is always modest when compared to rodent hepatocytes [11, 14, 15]. This may be due to the >ten-fold lower expression level of PPAR α in human liver compared to rodent liver [10]. Thus, PPAR α mediates CYP4A11 induction in humans, but exerts a more constrained effect than is observed in rodents.

The CYP4F subfamily—CYP4F2, CYP4F3A, CYP4F3B, CYP4F8, CYP4F11, CYP4F12, and CYP4F22 are the seven members of the human CYP4F subfamily. These enzymes ω -hydroxylate medium-chain (C10-C16), long-chain (C17-C21), and very long chain (C22-C26) fatty acids that are saturated, unsaturated, and/or branched. The rat has four members of the CYP4F subfamily (CYP4F1, 4, 5, and 6) and the mouse possesses five CYP4F enzymes (CYP4F13, 14, 15, 16, and 18) [16].

Human CYP4F3A is expressed only in neutrophils and is involved in the inflammatory process. This enzyme ω -hydroxylates leukotriene-B4 (LTB4), a pro-inflammatory eicosanoid, to produce a less active compound, 20-hydroxy-LTB4. CYP4F3A and CYP4F3B enzymes are structurally identical except for residues 67–114. Alternative splicing of the *CYP4F3* gene leads to insertion of exon 4 into CYP4F3A RNA and exon 3 into CYP4F3B RNA to generate proteins with unique functional activities and tissue expression. CYP4F8 is expressed mainly in seminal vesicles, testis, and epidermis, where it metabolizes prostaglandins and eicosanoids [17]. CYP4F22 is expressed in epidermis and

also metabolizes eicosanoids [18, 19]. CYP4F2, CYP4F3B, CYP4F11, and CYP4F12 are mainly expressed in liver and kidney [4, 7, 8, 20, 21]. These four enzymes share a high degree of sequence homology, making it difficult to determine the absolute amount of each enzyme in tissue using immunoquantitation with polyclonal antibodies. Hirani *et al.* developed a selective peptide antibody for CYP4F2 that reportedly did not have affinity for other CYP4F enzymes [22], and found that CYP4F2 enzyme content varied widely in human liver (ranging from 0–80 pmoles/mg microsomal protein) with an average of 18 ± 20 pmol/mg ($n = 29$). CYP4F2 content in kidney averaged 4 ± 4 pmol/mg and ranged from 0–11 pmol/mg ($n = 10$). McDonald *et al.* found CYP4F2 specific content to range from 3–11 pmoles/mg pooled liver microsomal protein and to vary according to the presence or absence of the common Val433Met polymorphism that is observed in ~30% of Caucasians [23]. The specific enzyme content of CYP4F3B, CYP4F11, and CYP4F12 in the liver is presently unknown, however, total CYP4F content in the liver is reported to range from 18–128 pmol/mg liver microsomal protein [24]. This represents a significant amount of hepatic P450 when compared to the major drug metabolizing P450 enzymes. Recently, tryptic digestion followed by mass spectrometry with target peptide selection has been used to provide absolute quantitation of P450 enzymes in pooled human liver microsomes. CYP3A4, CYP1A2, CYP2C9, and CYP2D6 specific content was 64, 18, 80, and 12 pmol/mg respectively [25]. This approach may be useful for absolute quantitation of CYP4 enzymes in the liver (and other tissues) as it does not depend on the availability of monospecific antibodies.

Figure 2 depicts the CYP4F gene cluster on chromosome 19. Regulation of CYP4F enzymes, unlike those in the CYP4A sub-family, is not controlled by the nuclear receptor PPAR α . Instead, statin drugs such as lovastatin and mevastatin induce CYP4F2 mRNA and protein in both HepG2 cells and primary human hepatocytes [26]. In these studies, statins selectively increased CYP4F2 mRNA by 3-fold in HepG2 cells and 5-fold in hepatocytes, with no increase in CYP4F3B mRNA. Immunoquantitation with an antibody that recognized both CYP4F2 and CYP4F3B showed a 2-fold increase in protein in HepG2 cells and a 3-fold increase in hepatocytes. Induction was mediated by the DNA-binding protein, sterol regulatory element-binding protein (SREBP), which regulates transcription of genes involved in cholesterol and fatty acid synthesis. In the endoplasmic reticulum (ER) SREBP is bound to the SREBP cleavage-activating protein (SCAP). When cell sterol levels are high, Insig (insulin-induced gene) protein binds to the SCAP-SREBP complex and retains it in the ER. Upon sterol depletion, which occurs after statin treatment, the SCAP-SREBP complex is transferred to the Golgi where SREBP is proteolysed and the active portion of the protein moves to the nucleus and regulates gene transcription [27]. Statin drugs have two main mechanisms for decreasing cholesterol from circulation; decreased biosynthesis of cholesterol by inhibiting the enzyme, HMGCoA reductase, and enhanced cholesterol uptake into the hepatocyte by increased expression of the low density lipoprotein (LDL) receptor. Thus, the *CYP4F2* gene may be transactivated by SREBP in order to clear the increased flux of triglycerides and other fatty acids resulting from increased LDL import into the liver cell. CYP4F2 expression is also induced in hepatocytes and HepG2 cells by AMP-activated protein kinase (AMPK) activators [28]. In these studies CYP4F2 mRNA was increased (2.5-13-fold) by three indirect AMPK activators: AICAR, genistein, and resveratrol.

Inhibitors of AMPK were shown to potentiate this increase in expression. In contrast, CYP4F3, CYP4A11, and CYP4F11 mRNAs were not affected by any AMPK activator in HepG2 cells, and CYP4F12 mRNA was only modestly increased (2-fold) upon treatment with genistein. Immunoquantitation with an antibody recognizing CYP4F2 and CYP4F3B, but not CYP4F11 or CYP4F12, revealed 1.6-1.9-fold increases in protein in HepG2 cells upon treatment with the AMPK activators. The physiological reason for AMPK-mediated CYP4F2 induction is unclear; however, it may be a defensive response to high levels of fatty acids in the mitochondria that result in lipotoxicity and hindered ATP synthesis [29]. AMPK is activated when AMP/ATP ratios in the cell are high in order to increase ATP production and reduce its utilization. Upon activation, AMPK phosphorylating activity is increased 1000-fold, and phosphorylation of, yet to be defined, transcription factors may be responsible for transactivation of the *CYP4F2* gene. Induction of CYP4F2 could help metabolically clear excessive free fatty acids from the cell and increase mitochondrial efficiency. Regardless, AMPK activation occurs when cellular ATP levels fall. This can arise due to numerous cellular stress factors, which may contribute to the large inter-individual variation of CYP4F2 protein levels observed in human liver microsomes.

CYP4F3B mRNA is induced in HepaRG cells by prostaglandin A1 in a concentration-dependent manner (1.8-6-fold after 1–40 μ M PGA1 treatment) [30]. In contrast, basal CYP4F3A mRNA was low in the cells and was not inducible by PGA1. Other prostaglandins and eicosanoids tested did not increase CYP4F3B mRNA. Western blotting with an antibody that recognized CYP4F3B, but not CYP4F3A, showed that protein levels were increased 3-fold in HepaRG cells after exposure to PGA1 (20 μ M). The antibody that was used was raised against the amino acids encoded by the CYP4F3B-specific exon of the *CYP4F3* gene. This amino acid sequence (residues 67–114) shares 27%, 83%, and 66% sequence identity with CYP4F3A, CYP4F2, and CYP4F11, respectively. Cross reactivity with CYP4F2 was not assessed and CYP4F2 mRNA was not measured, therefore it is unclear whether CYP4F3B alone or both CYP4F3B and CYP4F2 were induced by PGA1. PGA1 is a signaling eicosanoid that causes renal vasodilation, promotes sodium excretion in the kidney, and is an anti-hypertensive agent. PGA1 also has been shown to inhibit growth of some tumor cells. Therefore, regulation of CYP4F3B by PGA1 may be yet another factor in 20-HETE homeostasis and regulation of vascular tone.

Expression regulation of CYP4F3A differs markedly from that of CYP4F3B. CYP4F3A mRNA and associated LTB-4 ω -hydroxylase activity is induced by all-trans retinoic acid (ATRA) in HL60 cells [31]. Benzene is toxic to bone marrow and since CYP4F3A is expressed selectively in myeloid tissue, regulation of this enzyme by benzene and its metabolites has been studied by several groups. CYP4F3A protein was induced *in vivo* in white blood cells of patients with occupational benzene exposure [32], and CYP4F3A mRNA and protein levels were markedly induced in HL-60 cells by phenol, a metabolite of benzene, and by ATRA. Benzene itself showed only modest induction of CYP4F3A in this cell line.

CYP4F11 is regulated by the nuclear receptor, RXR, in HaCaT cells and HepG2 cells. The RXR selective chemical agonist, LG268 (aka LG100268), induced CYP4F11 mRNA (12-fold) and protein in HaCaT cells, whereas the RAR selective chemical agonist, TTNBP,

decreased mRNA (3-fold) and protein [33]. In the same cell line, the cytokines, TNF α and IL-1 β , induced CYP4F11 *via* a JNK, AP-1, and RXR/RAR mediated signaling pathway. In HepG2 cells, TNF α also induced CYP4F11. Enzyme induction was greater when a NF- κ B translocase inhibitor (IMD0354) was added to the TNF α treatment. Thus, NF- κ B may negatively regulate CYP4F11 expression [34]. However, cross-reactivity of the antibody used for western blotting was not assessed in these studies and other CYP4F enzymes may also have been induced.

CYP4F12 expression appears to be controlled by the pregnane-X receptor (PXR) [35]. In a chromatin immunoprecipitation (ChIP) study, a PXR binding site was found on the *CYP4F12* gene. Furthermore, siRNA knockdown of PXR in human hepatocytes resulted in an ~80% decrease CYP4F12 basal mRNA. Thus, CYP4F12 protein would be expected to be induced by PXR agonists such as rifampicin. This is significant because CYP4F12 is involved in xenobiotic metabolism. Indeed, drugs such as ebastine and terfenadine are CYP4F12 substrates, and induction by PXR ligands might be expected to lead to drug-drug interactions.

Regulation of CYP4F8 and CYP4F22 expression have not been investigated to date.

Other CYP4 subfamilies—CYP4B1, the only member of the CYP4B subfamily, is expressed at the RNA level at high levels in human lung, but is catalytically inactive due to a mutation (Pro427Ser) in the meander region of the protein [36]. However, CYP4B1 is functional in other mammals, many of which are used to model human disease. The potential species-difference in metabolism is important because animal forms of CYP4B1 metabolize some procarcinogens and xenobiotics, in addition to short (C7-C10) chain fatty acids [37, 38].

The remaining CYP4 subfamilies each have one member: CYP4V2, CYP4X1, and CYP4Z1. These enzymes have been classified as orphan P450s, as their substrates and functions *in vivo* are poorly defined. CYP4V2 is known to hydroxylate medium chain fatty acids *in vitro* [39]. The enzyme is expressed ubiquitously and mutations in the *CYP4V2* gene are associated with the rare eye disease; Bietti's crystalline dystrophy. CYP4Z1 is expressed solely in mammary tissue, and can be induced by dexamethasone in breast cancer cell lines [40]. CYP4X1 is expressed mostly in aorta and trachea, however, it is also present in liver, kidney, and a few other extrahepatic tissues. In HepG2 cells, CYP4X1 was induced by the potent PPAR α agonist, WY17643, although basal levels were undetectable by western blotting [40]. Known inducers of CYP4 enzymes are summarized in Table 1.

CYP4 selective chemical inhibitors have been developed over the past two decades in order to study CYP4 specific reactions, such as 20-HETE formation, and to target CYP4 enzymes for possible therapeutic benefit (Table 2). The first CYP4 specific inhibitors were fatty acid derivatives containing a terminal acetylene moiety [41]. These inhibitors were designed to mimic typical fatty acid substrates for the CYP4s and to cause mechanism-based inactivation. Terminal di-bromo fatty acid analogues were developed as reversible, competitive CYP4 inhibitors. The introduction of a sulfated group in place of the carboxylic acid increased the *in vivo* half-life by eliminating the potential for rapid degradation of the

inhibitor *via* β -oxidation. 17-Octadecynoic acid (17-ODYA) has emerged as the most popular CYP4 inhibitor used *in vitro* and *in vivo* studies, however its selectivity for CYP4 inhibition is poor, as IC₅₀ values for arachidonic acid epoxigenase (non-CYP4 reaction) and terminal hydroxylase activities were similar (3 μ M and 5 μ M, respectively) [8].

Formamidoximes related to HET0016 (N-hydroxy-N'-(4-n-butyl-2-methylphenyl)-formamidine) are a newer class of CYP4 inhibitors that are more potent and selective than the terminally substituted fatty acids. HET0016, which does not resemble typical fatty acid substrates, was discovered by a drug development program searching for 20-HETE synthase inhibitors to be used as a treatment for renal hypertension. HET0016 selectively inhibited CYP4-mediated formation of 20-HETE in human renal microsomes with an IC₅₀ value of <10 nM [42, 43], while inhibiting P450-mediated epoxigenase activity and cyclooxygenase activity with IC₅₀ values of 2.8 μ M and 2.3 μ M, respectively. HET0016 appears to be a potent pan-CYP4 inhibitor, inhibiting CYP4A11, CYP4F2, CYP4F3B, CYP4V2, and CYP4B1 with IC₅₀ values of 42 nM, 125 nM, 100 nM, 38 nM, and 37 nM, respectively [39, 44, 45]. However, caution should be used when using this inhibitor for selective CYP4 inhibition in *in vivo* studies. Indeed, the IC₅₀ for CYP3A4 and CYP2D6 inhibition are >50 μ M, however the IC₅₀ for CYP2C9, CYP2C19, and CYP1A2 inhibition are considerably lower (4.2 μ M, 272 nM, and 461 nM, respectively) [46]. TS-011 (N-(3-chloro-4-morpholin-4-yl)phenyl-N'-hydroxyimidoformamide) is a related formamidoxime that is just as potent and selective as HET0016 for inhibition of 20-HETE formation, but has the added advantage of increased water solubility [47]. Currently, there are no isoform-selective inhibitors for any CYP4 enzyme.

CYP4 Enzymes as Drug Targets

Since the emergence of seemingly pan-CYP4 chemical inhibitors such as HET0016, as well as CYP4 inducing agents, investigators have been better able to probe the role of 20-HETE in the pathophysiology of numerous diseases, including hypertension, stroke, and cancer. Reduction in 20-HETE synthesis *via* inhibition of CYP4 enzymes has been promoted as a promising therapeutic strategy for these conditions [51–54]. Additionally, CYP4 enzymes may be viable drug targets in metabolic disorders, such as X-ALD and Refsum disease, which result from compromised clearance of fatty acids, as discussed below.

Inhibition of CYP4 enzymes and 20-hydroxyeicosatetraenoic acid (20-HETE) biosynthesis

The oxidation of arachidonic acid by cyclooxygenases, lipoxygenases, and P450 enzymes generates eicosanoids that possess a wide spectrum of biological activity and regulate complex signaling processes such as inflammation [55], platelet aggregation [56, 57], and vascular and bronchiolar tone [58, 59]. CYP4 enzymes catalyze the formation of the key signaling eicosanoid, 20-HETE, which is the ω -hydroxylated metabolite of arachidonic acid [8]. As discussed in detail below, inhibition of CYP4 enzymes, and thus of 20-HETE biosynthesis, has been suggested as a therapeutic strategy for hypertension, stroke, and some cancers because 20-HETE may contribute to the pathogenesis of these conditions [51–54].

20-HETE can be produced from arachidonic acid by human CYP4A11, CYP4F2, CYP4F11, CYP4F3A and CYP4F3B [47, 60, 61]. *In vitro* catalytic efficiencies can vary widely for this

reaction [5, 7, 8], which may reflect the degree of difficulty involved in measuring accurate kinetic constants for very lipophilic endogenous compounds residing mostly in biological membranes. 19-HETE is also a minor metabolite produced by these enzymes from arachidonic acid, but it is often ignored or mistakenly measured as 20-HETE because it is difficult to distinguish 19- and 20-HETE with HPLC separation gradients shorter than 30 minutes [7, 8]. Therefore, when interpreting reports of 20-HETE levels in urine, tissue, or enzyme incubations, it is important to evaluate whether the assay achieved chromatographic separation because these two metabolites are typically monitored by the same mass spectral multiple-reaction monitoring (MRM) transition. Pharmacologically, this is critical because 19-HETE is an antagonist of 20-HETE and is the major arachidonic acid metabolite formed by other P450 enzymes such as CYP2E1 [62–64].

Hypertension—20-HETE has pro- and anti-hypertensive effects in numerous studies conducted in rat and mouse models of hypertension. The extensive body of literature investigating the role of 20-HETE in animal models of hypertension has been thoroughly reviewed elsewhere [51, 65, 66]. It is clear from these studies that 20-HETE plays a complex role in blood pressure regulation, and the balance between pro- and anti-hypertensive effects is critically dependent on the extent and site of 20-HETE biosynthesis [65, 66]. Moreover, the mouse and rat possess many CYP4 enzymes that generate 20-HETE, each with expression patterns and modes of regulation that may differ from human CYP4 enzymes, making translation of results from *in vivo* animal studies to the human situation particularly difficult.

Generally speaking, 20-HETE is a potent vasoconstrictor in the vasculature, including arterioles in the kidney [67], brain [68–70], and heart [71, 72], and so is considered to be pro-hypertensive in this tissue. 20-HETE activates a number of kinase pathways that contribute to vascular tone regulation and blocks calcium-dependent potassium (K_{Ca}) channels that lead to increased calcium entry through L-type Calcium channels in the vascular smooth muscle cell. Increased Ca^{2+} in these cells causes smooth muscle contraction and vasoconstriction. However, in the kidney 20-HETE has an *anti*-hypertensive effect. This appears to be due to inhibition of Na^+ reabsorption in the proximal tubule of the nephron through activation of protein kinase C that phosphorylates renal $Na^+K^+ATPase$, causing decreased sodium transport through this channel. In the thick ascending loop of Henle (TALH), 20-HETE blocks other ion channels, namely $Na^+K^+2Cl^-$ and 70pS K^+ , leading to reduced passive reabsorption of Na^+ in this region of the nephron. Thus, in the nephron, 20-HETE has a natriuretic effect leading to less water reabsorption, thereby lowering blood pressure [51, 65, 73].

In humans, genetic association studies linking variants of CYP4A11 and CYP4F2 with elevated blood pressure have highlighted the complexity inherent in having multiple CYP4 enzymes contributing to the biosynthesis of 20-HETE. A T8590C single nucleotide polymorphism (SNP) in the *CYP4A11* gene (rs1126742) results in an F434S amino acid substitution that both decreases V_{max} and catalytic efficiency for 20-HETE formation when compared to the wild type enzyme [5]. In two separate populations ($n=512$ and $n=1538$), individuals who were homozygous or heterozygous carriers of the 8590C allele had an increased risk of hypertension (odds ratio of 2.31 and 1.21 for each cohort, respectively)

compared to 8590T homozygotes. The functional deficit of the variant enzyme should result in decreased 20-HETE production in tissue where CYP4A11 is expressed, suggesting that the natriuretic and anti-hypertensive effects of 20-HETE at the level of the nephron drive the genetic association. Correspondingly, in the *CYP4F2* gene a C1347T SNP (rs2108622) results in a V433M amino acid substitution and a T84G SNP (rs3093105) results in a W12G amino acid substitution. The V433M mutant, expressed in insect cells, exhibited a decreased *k_{cat}* for 20-HETE formation from 0.08 min⁻¹ to 0.03 min⁻¹ at a concentration of 42 μM arachidonic acid [74], and there was no change in enzyme activity of the W12G mutant. Surprisingly, there are no published data on *K_m*, *V_{max}*, or catalytic efficiency for these CYP4F2 mutants and 20-HETE formation, so it cannot be conclusively stated that they are either gain or loss of function polymorphisms. A study (n=235) that measured the effects of both loss of function polymorphisms in *CYP4A11* (rs1126742) and *CYP4F2* (rs2108622) on blood pressure and urinary 20-HETE levels provide a less clear conclusion. In carriers of the *CYP4A11* 8590C SNP, there was a decrease in urinary 20-HETE, but no effect on blood pressure. In carriers of the CYP4F2 1347T SNP, there was an unexpected increase in urinary 20-HETE as well as an increase in blood pressure. However, a 50% increase in enzyme activity above wild-type levels for 20-HETE production by the CYP4F2 W12G mutant was later reported, suggesting a gain of function polymorphism that may have been overlooked in the earlier study. A different study looked at mutations in haplotypes in the regulatory regions of the *CYP4F2* gene and found an association between a common genetic variant of one haplotype (Hap I: rs3093098, rs3093100, rs3093103, and rs3093105) and increased risk of hypertension along with elevated urinary 20-HETE levels [75]. The authors propose that differences in NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) binding to the regulatory region may be the cause for this association. Another study in Swedes (n=6002) found subjects with the CYP4A11 F434S mutation (rs1126742) had higher blood pressure and hypertension prevalence, corroborating earlier findings. However, the same study found increased blood pressure and a slight trend towards increased hypertension risk only in males with the CYP4F2 V433M mutation, with no effect in females [76]. Collectively, these genetic association studies suggest that both CYP4A11 and CYP4F2 contribute to 20-HETE biosynthesis and blood pressure regulation *in vivo* in humans, but the precise mechanisms are difficult to unravel. A further complication is that no studies have yet been reported in humans concerning the effect on blood pressure of polymorphisms in CYP4F3B and CYP4F11, two other potentially important 20-HETE synthases.

Clearly, the role of 20-HETE in the pathophysiology of hypertension is complex, since it is a natriuretic and anti-hypertensive mediator in the nephron of the kidney and a vasoconstrictor and pro-hypertensive eicosanoid in the vascular smooth muscle cells. Additionally, other eicosanoids, such as EETs and diHETEs, contribute to vascular tone regulation and introduce additional complications [77]. Nevertheless, numerous animal studies have provided strong evidence overall that indicates that inhibition of CYP4 enzymes and 20-HETE formation is a promising route to hypertension therapy.

Hemorrhagic and Ischemic Stroke—There are a number of studies that have investigated the potential for CYP4 inhibition as a treatment following stroke to reduce brain

damage. The rationale here is that 20-HETE is a vasoconstrictor in cerebral arteries *in vitro* and *in vivo* [69]. In the brain, oxygen deprivation to tissue occurs during both hemorrhagic stroke and ischemic stroke. During ischemic stroke caused by prolonged blockage of cerebral arteries, blood flow to tissue is restricted and neurons die, resulting in an infarct. The size of the resulting infarct reflects the extent of damage to brain tissue. During subarachnoid hemorrhage (SAH) caused by ruptured aneurysm or head injury, there is an acute drop in cerebral blood flow resulting in ischemia. After SAH in rats, inhibitors of 20-HETE synthesis, such as HET0016 and 17-ODYA, decreased 20-HETE levels in cerebral spinal fluid (CSF) and prevented the acute fall in cerebral blood flow [44]. However, the inhibitors used in this study were likely not CYP4 selective. As mentioned above, 17-ODYA inhibits EET and HETE formation at similar IC_{50} values *in vitro*, and the dose of HET0016 given to the rats (10 mg/kg) was rather large and perhaps sufficiently high to inhibit other P450 enzymes, such as isoforms from the CYP2C sub-family. However, a subsequent study found that TS-011 also prevented the drop in cerebral blood flow and rise in 20-HETE levels in the CSF after SAH, and the dose was more appropriately reflective (0.01–1 mg/kg) of a potent enzyme inhibitor [47]. After ischemic stroke in rats, CYP4 inhibition by TS-011 treatment decreased 20-HETE production and caused a 35% decrease in infarct size. Furthermore, in a different rat model of ischemic stroke (transient occlusion of the middle cerebral artery), there was a 55% and 65% decrease in infarct volume size after TS-011 and HET0016 administration, respectively [78]. This latter study measured HETE and EET levels in brain tissue and found an 80% decrease in 20-HETE after inhibitor dosing with no effect on EET levels. The HPLC/MS/MS method also enabled analytical discrimination of 19- and 20-HETE, although 19-HETE was not quantitated. Together, these studies indicate a potential for targeting CYP4 enzymes in the cerebral vasculature with the aim of reducing 20-HETE production, decreasing vasoconstriction, and thus increasing blood flow to ischemic regions of the brain after stroke. Additionally, inhibitors like TS-011 may cross the blood brain barrier and affect 20-HETE biosynthesis in the neuron, as well as the cerebral vasculature. Because 20-HETE is involved in numerous signal transduction pathways and affects ion transport across cell membranes, there may be additional mechanisms for the protective effects of CYP4 inhibitors after ischemia. However, it is not clear which CYP4 enzymes are expressed and produce 20-HETE in the neuron, and a non-CYP4 brain P450, CYP2U1, has been shown to form both 19- and 20-HETE [79]. It is also not known if HET0016 or TS-011 inhibit CYP2U1. Therefore, more work must be done to validate this alternative mechanism of action and clarify whether CYP4 inhibitors could be viable drugs for stroke victims.

Cancer—Angiogenesis is the growth of new blood vessels from vascular endothelial cells and is mediated by a number of peptide growth factors and cytokines. 20-HETE is a pro-angiogenic signaling eicosanoid and mediates neovascularization of various cancers through activation of vascular endothelial growth factor (VEGF) [80]. Additionally, CYP4 enzymes that biosynthesize 20-HETE are up-regulated in some cancers [54]. Remarkable decreases in tumor size after administration of HET0016 to animal models have been observed [81]. *In vitro*, HET0016 inhibited brain tumor cell proliferation (U251 glioma cells) in a dose dependent manner. In a rat model of a highly aggressive brain tumor, HET0016 dosed daily for 2 weeks (10 mg/kg/day) decreased the size of the tumor by 80%, decreased

neovascularization by ~50%, and increased mean survival time of the animal from 17 to 22 days [82, 83]. Thus, CYP4 appears to be a promising drug target for inhibition in rat solid brain tumors. Additionally, HET0016 inhibited growth of human renal adenocarcinoma cell lines (786-O and 769-P cell lines) *in vitro*. An adenocarcinoma is cancer of the epithelial cells originating in a glandular tissue. Cells were implanted in an ectopic mouse model of cancer and administration of a 20-HETE antagonist, 20-5(Z),14(Z)-hydroxyeicosadienoic acid (WIT002), decreased the tumor size by 84% [84]. The study did not report similar results with any CYP4 inhibitor, and so more work is needed before it can be concluded that 20-HETE reduction by CYP4 inhibition is a possible therapeutic modality for renal adenocarcinoma.

Induction of CYP4 enzymes in fatty acid clearance disorders

Fatty acid oxidation and clearance overview—Most fatty acids (FAs) are cleared by the well-characterized elimination pathway, mitochondrial or peroxisomal β -oxidation (Figure 3). Acyl coA-esters of FAs are transferred into the mitochondria and undergo four transformations performed by four separate enzymes: dehydrogenation, hydration, a second dehydrogenation, and finally cleavage of the acyl-coA group. This cascade yields a FA that is two carbons shorter than the original substrate. Generally, short, medium and long chain FAs undergo β -oxidation in the mitochondria by enzymes with specificities for each chain length [85]. Very long chained FAs (>C22) are β -oxidized in the peroxisomes and then may be transferred to the mitochondria where further rounds of β -oxidation occur [86]. FAs that are branched or substituted at the β -carbon must first undergo α -oxidation, and this only occurs only in the peroxisome [87]. ω -Oxidation is a third route of FA degradation, accounting for only a small percentage of the total clearance pathway under normal conditions. As noted in earlier sections, CYP4 enzymes ω -hydroxylate FAs, and alcohol dehydrogenases (ADH), aldehyde dehydrogenases (ALDH), as well as other P450 enzymes, perform further oxidative steps to yield a dicarboxylic acid [88]. The dicarboxylic acid can then be esterified to an acyl coA-ester and transferred to the peroxisome to be degraded *via* β -oxidation.

X-linked adrenoleukodystrophy (X-ALD)—X-ALD is an inherited disorder characterized by an increase in very long chain fatty acids (VLCFA) in plasma and tissue resulting in demyelination of neurons. The incidence of X-ALD is 1 in 20,000 males and treatments are limited to bone marrow transplantation and gene therapy. Mutations in the *ABCD1* (ATP-binding cassette, subfamily D, member 1) gene on the X chromosome leads to a dysfunctional protein normally responsible for transporting VLCFAs into the peroxisome for degradation. Increasing ω -hydroxylation of excess VLCFAs to generate dicarboxylic acids via *CYP4F2* induction has been suggested as a therapeutic strategy for X-ALD patients [88, 89]. Very long chain dicarboxylic acids are transported into the peroxisome by a different transporter than their monocarboxylic acid counterparts. Thus, *CYP4F2* induction might be an alternative pathway to clear VLCFAs from the liver cell and thus the systemic circulation. Lovastatin, a *CYP4F2* inducer, was reported to lower VLCFAs in plasma of patients and has been used as an off-label therapy for this disease [90, 91]. However, a recent placebo-controlled trial showed no benefit of lovastatin treatment in X-ALD patients [92].

Refsum Disease—Adult Refsum disease is an autosomal recessive disorder characterized by an increase in phytanic acid in plasma and tissue resulting in neural defects. Loss of function mutations in either the Peroxin 7 (*PEX7*) gene or the phytanoyl-CoA 2-hydroxylase (*PAHX aka PHYH*) gene that lead to impaired α -oxidation of phytanic acid underlie adult Refsum. Phytanic acid has a methyl group in the β -position, necessitating α -oxidation in the peroxisome before it can undergo β -oxidation in the mitochondria. Phytanoyl-coA 2-hydroxylase is responsible for the hydroxylation step of phytanoyl-coA during α -oxidation. The *PEX7* gene codes for the PTS2 (peroxisomal targeting signal 2) receptor, which binds to some proteins synthesized in the cytoplasm and directs them to the peroxisome. A dysfunctional PTS2 receptor results in faulty transport of phytanoyl-coA 2-hydroxylase from the cytoplasm to the peroxisome. In patients with Refsum disease, phytanic acid plasma concentrations have been reported to increase from $<10\ \mu\text{M}$ to as high as 1.3 mM [93]. CYP4 mediated ω -hydroxylation is responsible for only a small percentage of the clearance pathway under normal conditions, but may be an alternate route to phytanic acid clearance in the disease state. The catalytic efficiencies in human P450 Supersomes for ω -hydroxylation of phytanic acid are CYP4F3A > CYP4F3B > CYP4F2 > CYP4A11 [94]. Consequently, induction of CYP4F/CYP4A enzymes has been proposed as a therapeutic route to treat Refsum disease [87, 95].

Concluding Remarks

The CYP4 enzyme family represents a multifunctional component of human biochemistry and physiology, with member enzymes expressed in nearly every tissue. CYP4 enzymes are responsible for activation and deactivation of numerous eicosanoid signaling pathways and, therefore, present a viable drug target for several diseases. However, blanket inhibition of CYP4 enzymes as a therapeutic strategy is far from ideal. Future work should focus on development of more selective molecular tools that enable chemical induction and inhibition of specific sub-family members, and eventually delivery of a drug to the target enzyme in specific cell types and tissues. Thus, while the CYP4 enzyme family appears a feasible drug target, much remains to be learned about the role of these enzymes in the pathophysiology of disease before a therapeutically useful entity emerges from this field.

List of Abbreviations

CYP4	Cytochrome P450 4
20-HETE	20-Hydroxyeicosatetraenoic Acid
ER	endoplasmic reticulum
SREBP	sterol regulatory element binding protein
LDL	low density lipoprotein
PXR	pregnane-X receptor
PGA1	prostaglandin A1
ATRA	all- <i>trans</i> retinoic acid

17-ODYA	17-octadecynoic acid
EET	epoxyeicosatetraenoic acid
FA	fatty acid
VLCFA	very long chain fatty acid
SAH	subarachnoid hemorrhage
X-ALD	X-linked adrenoleukodystrophy
InsIG	insulin induced gene
VEGF	vascular endothelial growth factor
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
TNFα	tumor necrosis factor-alpha
AMP	adenosine monophosphate
AMPK	AMP-activated protein kinase
SCAP	SREBP cleavage activating protein
PTS2	peroxisomal targeting signal 2

References

1. Hoch U, Ortiz De Montellano PR. Covalently linked heme in cytochrome p450a fatty acid hydroxylases. *The Journal of biological chemistry*. 2001; 276(14):11339–11346. [PubMed: 11139583]
2. Henne KR, Kunze KL, Zheng YM, Christmas P, Soberman RJ, Rettie AE. Covalent linkage of prosthetic heme to CYP4 family P450 enzymes. *Biochemistry*. 2001; 40(43):12925–12931. [PubMed: 11669629]
3. Bylund J, Hildestrand M, Ingelman-Sundberg M, Oliw EH. Identification of CYP4F8 in human seminal vesicles as a prominent 19-hydroxylase of prostaglandin endoperoxides. *The Journal of biological chemistry*. 2000; 275(29):21844–21849. [PubMed: 10791960]
4. Bylund J, Bylund M, Oliw EH. cDNA cloning and expression of CYP4F12, a novel human cytochrome P450. *Biochemical and biophysical research communications*. 2001; 280(3):892–897. [PubMed: 11162607]
5. Gainer JV, Bellamine A, Dawson EP, Womble KE, Grant SW, Wang Y, Cupples LA, Guo CY, Demissie S, O'Donnell CJ, Brown NJ, Waterman MR, Capdevila JH. Functional variant of CYP4A11 20-hydroxyeicosatetraenoic acid synthase is associated with essential hypertension. *Circulation*. 2005; 111(1):63–69. [PubMed: 15611369]
6. Dierks EA, Zhang Z, Johnson EF, de Montellano PR. The catalytic site of cytochrome P450A11 (CYP4A11) and its L131F mutant. *The Journal of biological chemistry*. 1998; 273(36):23055–23061. [PubMed: 9722531]
7. Powell PK, Wolf I, Jin R, Lasker JM. Metabolism of arachidonic acid to 20-hydroxy-5,8,11, 14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. *The Journal of pharmacology and experimental therapeutics*. 1998; 285(3):1327–1336. [PubMed: 9618440]
8. Lasker JM, Chen WB, Wolf I, Bloswick BP, Wilson PD, Powell PK. Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of Cyp4F2 and Cyp4A11. *The Journal of biological chemistry*. 2000; 275(6):4118–4126. [PubMed: 10660572]

9. Johnson EF, Palmer CN, Griffin KJ, Hsu MH. Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. *The FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 1996; 10(11):1241–1248.
10. Johnson EF, Hsu MH, Savas U, Griffin KJ. Regulation of P450 4A expression by peroxisome proliferator activated receptors. *Toxicology*. 2002;181–182. 203–206. [PubMed: 11893417]
11. Lawrence JW, Li Y, Chen S, DeLuca JG, Berger JP, Umbenhauer DR, Moller DE, Zhou G. Differential gene regulation in human versus rodent hepatocytes by peroxisome proliferator-activated receptor (PPAR) alpha. PPAR alpha fails to induce peroxisome proliferation-associated genes in human cells independently of the level of receptor expression. *The Journal of biological chemistry*. 2001; 276(34):31521–31527. [PubMed: 11418601]
12. Cattley RC, DeLuca J, Elcombe C, Fenner-Crisp P, Lake BG, Marsman DS, Pastoor TA, Popp JA, Robinson DE, Schwetz B, Tugwood J, Wahli W. Do peroxisome proliferating compounds pose a hepatocarcinogenic hazard to humans? *Regulatory toxicology and pharmacology: RTP*. 1998; 27(1 Pt 1):47–60.
13. Savas U, Machemer DE, Hsu MH, Gaynor P, Lasker JM, Tukey RH, Johnson EF. Opposing roles of peroxisome proliferator-activated receptor alpha and growth hormone in the regulation of CYP4A11 expression in a transgenic mouse model. *The Journal of biological chemistry*. 2009; 284(24):16541–16552. [PubMed: 19366684]
14. Alvergnas M, Richert L, Blanchard N, Abadie C, Heyd B, Manton G, Gallemann D, Martin H. Regulation of CYP4A expression by bezafibrate in primary culture of rat and human hepatocytes: interspecies difference and influence of N-acetylcysteine. *Toxicology in vitro: an international journal published in association with BIBRA*. 2009; 23(7):1259–1267. [PubMed: 19647067]
15. Raucy JL, Lasker J, Ozaki K, Zoleta V. Regulation of CYP2E1 by ethanol and palmitic acid and CYP4A11 by clofibrate in primary cultures of human hepatocytes. *Toxicological sciences: an official journal of the Society of Toxicology*. 2004; 79(2):233–241. [PubMed: 15056802]
16. Cui X, Kawashima H, Barclay TB, Peters JM, Gonzalez FJ, Morgan ET, Strobel HW. Molecular cloning and regulation of expression of two novel mouse CYP4F genes: expression in peroxisome proliferator-activated receptor alpha-deficient mice upon lipopolysaccharide and clofibrate challenges. *The Journal of pharmacology and experimental therapeutics*. 2001; 296(2):542–550. [PubMed: 11160642]
17. Stark K, Torma H, Cristea M, Oliw EH. Expression of CYP4F8 (prostaglandin H 19-hydroxylase) in human epithelia and prominent induction in epidermis of psoriatic lesions. *Archives of biochemistry and biophysics*. 2003; 409(1):188–196. [PubMed: 12464258]
18. Nilsson T, Ivanov IV, Oliw EH. LC-MS/MS analysis of epoxyalcohols and epoxides of arachidonic acid and their oxygenation by recombinant CYP4F8 and CYP4F22. *Archives of biochemistry and biophysics*. 2010; 494(1):64–71. [PubMed: 19919823]
19. Kelly EJ, Nakano M, Rohatgi P, Yarov-Yarovoy V, Rettie AE. Finding homes for orphan cytochrome P450s: CYP4V2 and CYP4F22 in disease states. *Molecular interventions*. 2011; 11(2):124–132. [PubMed: 21540472]
20. Cui X, Nelson DR, Strobel HW. A novel human cytochrome P450 4F isoform (CYP4F11): cDNA cloning, expression, and genomic structural characterization. *Genomics*. 2000; 68(2):161–166. [PubMed: 10964514]
21. Christmas P, Jones JP, Patten CJ, Rock DA, Zheng Y, Cheng SM, Weber BM, Carlesso N, Scadden DT, Rettie AE, Soberman RJ. Alternative splicing determines the function of CYP4F3 by switching substrate specificity. *The Journal of biological chemistry*. 2001; 276(41):38166–38172. [PubMed: 11461919]
22. Hirani V, Yarovoy A, Kozeska A, Magnusson RP, Lasker JM. Expression of CYP4F2 in human liver and kidney: assessment using targeted peptide antibodies. *Archives of biochemistry and biophysics*. 2008; 478(1):59–68. [PubMed: 18662666]
23. McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: An explanation for altered warfarin dose in carriers of the V433M variant. *Molecular pharmacology*. 2009; 75(6):1337–1346. [PubMed: 19297519]
24. Jin Y, Zollinger M, Borell H, Zimmerlin A, Patten CJ. CYP4F enzymes are responsible for the elimination of fingolimod (FTY720): a novel treatment of relapsing multiple sclerosis. *Drug*

- metabolism and disposition: the biological fate of chemicals. 2011; 39(2):191–198. [PubMed: 21045201]
25. Kawakami H, Ohtsuki S, Kamiie J, Suzuki T, Abe T, Terasaki T. Simultaneous absolute quantification of 11 cytochrome P450 isoforms in human liver microsomes by liquid chromatography tandem mass spectrometry with in silico target peptide selection. *Journal of pharmaceutical sciences*. 2011; 100(1):341–352. [PubMed: 20564338]
 26. Hsu MH, Savas U, Griffin KJ, Johnson EF. Regulation of human cytochrome P450 4F2 expression by sterol regulatory element-binding protein and lovastatin. *The Journal of biological chemistry*. 2007; 282(8):5225–5236. [PubMed: 17142457]
 27. Goldstein JL, DeBose-Boyd RA, Brown MS. Protein sensors for membrane sterols. *Cell*. 2006; 124(1):35–46. [PubMed: 16413480]
 28. Hsu MH, Savas U, Lasker JM, Johnson EF. Genistein, resveratrol, and 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside induce cytochrome P450 4F2 expression through an AMP-activated protein kinase-dependent pathway. *The Journal of pharmacology and experimental therapeutics*. 2011; 337(1):125–136. [PubMed: 21205922]
 29. Weinberg JM. Lipotoxicity. *Kidney international*. 2006; 70(9):1560–1566. [PubMed: 16955100]
 30. Antoun J, Goulitquer S, Amet Y, Dreano Y, Salaun JP, Corcos L, Plee-Gautier E. CYP4F3B is induced by PGA1 in human liver cells: a regulation of the 20-HETE synthesis. *Journal of lipid research*. 2008; 49(10):2135–2141. [PubMed: 18566475]
 31. Mizukami Y, Sumimoto H, Takeshige K. Induction of cytochrome CYP4F3A in all-trans-retinoic acid-treated HL60 cells. *Biochemical and biophysical research communications*. 2004; 314(1):104–109. [PubMed: 14715252]
 32. Zhao Z, He X, Bi Y, Xia Y, Tao N, Li L, Ma Q. Induction of CYP4F3 by benzene metabolites in human white blood cells in vivo in human promyelocytic leukemic cell lines and ex vivo in human blood neutrophils. *Drug metabolism and disposition: the biological fate of chemicals*. 2009; 37(2):282–291. [PubMed: 19029204]
 33. Wang Y, Bell JC, Keeney DS, Strobel HW. Gene regulation of CYP4F11 in human keratinocyte HaCaT cells. *Drug metabolism and disposition: the biological fate of chemicals*. 2010; 38(1):100–107. [PubMed: 19812349]
 34. Bell JC, Strobel HW. Regulation of cytochrome P450 4F11 by nuclear transcription factor-kappaB. *Drug metabolism and disposition: the biological fate of chemicals*. 2012; 40(1):205–211. [PubMed: 22011441]
 35. Hariparsad N, Chu X, Yabut J, Labhart P, Hartley DP, Dai X, Evers R. Identification of pregnane-X receptor target genes and coactivator and corepressor binding to promoter elements in human hepatocytes. *Nucleic acids research*. 2009; 37(4):1160–1173. [PubMed: 19129222]
 36. Zheng YM, Henne KR, Charmley P, Kim RB, McCarver DG, Cabacungan ET, Hines RN, Rettie AE. Genotyping and site-directed mutagenesis of a cytochrome P450 meander Pro-X-Arg motif critical to CYP4B1 catalysis. *Toxicology and applied pharmacology*. 2003; 186(2):119–126. [PubMed: 12639503]
 37. Baer BR, Rettie AE. CYP4B1: an enigmatic P450 at the interface between xenobiotic and endobiotic metabolism. *Drug metabolism reviews*. 2006; 38(3):451–476. [PubMed: 16877261]
 38. Fisher MB, Zheng YM, Rettie AE. Positional specificity of rabbit CYP4B1 for omega-hydroxylation1 of short-medium chain fatty acids and hydrocarbons. *Biochemical and biophysical research communications*. 1998; 248(2):352–355. [PubMed: 9675139]
 39. Nakano M, Kelly EJ, Rettie AE. Expression and characterization of CYP4V2 as a fatty acid omega-hydroxylase. *Drug metabolism and disposition: the biological fate of chemicals*. 2009; 37(11):2119–2122. [PubMed: 19661213]
 40. Savas U, Hsu MH, Griffin KJ, Bell DR, Johnson EF. Conditional regulation of the human CYP4X1 and CYP4Z1 genes. *Archives of biochemistry and biophysics*. 2005; 436(2):377–385. [PubMed: 15797250]
 41. Kroetz DL, Xu F. Regulation and inhibition of arachidonic acid omega-hydroxylases and 20-HETE formation. *Annual review of pharmacology and toxicology*. 2005; 45:413–438.
 42. Sato M, Ishii T, Kobayashi-Matsunaga Y, Amada H, Taniguchi K, Miyata N, Kameo K. Discovery of a N'-hydroxyphenylformamidine derivative HET0016 as a potent and selective 20-HETE

- synthase inhibitor. *Bioorganic & medicinal chemistry letters*. 2001; 11(23):2993–2995. [PubMed: 11714595]
43. Miyata N, Taniguchi K, Seki T, Ishimoto T, Sato-Watanabe M, Yasuda Y, Doi M, Kametani S, Tomishima Y, Ueki T, Sato M, Kameo K. HET0016, a potent and selective inhibitor of 20-HETE synthesizing enzyme. *British journal of pharmacology*. 2001; 133(3):325–329. [PubMed: 11375247]
44. Kehl F, Cambj-Sapunar L, Maier KG, Miyata N, Kametani S, Okamoto H, Hudetz AG, Schulte ML, Zagorac D, Harder DR, Roman RJ. 20-HETE contributes to the acute fall in cerebral blood flow after subarachnoid hemorrhage in the rat. *American journal of physiology Heart and circulatory physiology*. 2002; 282(4):H1556–1565. [PubMed: 11893593]
45. Parkinson OT, Kelly EJ, Bezabih E, Whittington D, Rettie AE. Bioactivation of 4-Ipomeanol by a CYP4B enzyme in bovine lung and inhibition by HET0016. *Journal of veterinary pharmacology and therapeutics*. 2012; 35(4):402–405. [PubMed: 21919916]
46. Nakamura T, Sato M, Kakinuma H, Miyata N, Taniguchi K, Bando K, Koda A, Kameo K. Pyrazole and isoxazole derivatives as new, potent, and selective 20-hydroxy-5,8,11,14-eicosatetraenoic acid synthase inhibitors. *Journal of medicinal chemistry*. 2003; 46(25):5416–5427. [PubMed: 14640550]
47. Miyata N, Seki T, Tanaka Y, Omura T, Taniguchi K, Doi M, Bandou K, Kametani S, Sato M, Okuyama S, Cambj-Sapunar L, Harder DR, Roman RJ. Beneficial effects of a new 20-hydroxyeicosatetraenoic acid synthesis inhibitor, TS-011 [N-(3-chloro-4-morpholin-4-yl) phenyl-N'-hydroxyimido formamide], on hemorrhagic and ischemic stroke. *The Journal of pharmacology and experimental therapeutics*. 2005; 314(1):77–85. [PubMed: 15831442]
48. Shak S, Reich NO, Goldstein IM, Ortiz de Montellano PR. Leukotriene B₄ omega-hydroxylase in human polymorphonuclear leukocytes. Suicidal inactivation by acetylenic fatty acids. *The Journal of biological chemistry*. 1985; 260(24):13023–13028. [PubMed: 2997155]
49. Xu F, Straub WO, Pak W, Su P, Maier KG, Yu M, Roman RJ, Ortiz De Montellano PR, Kroetz DL. Antihypertensive effect of mechanism-based inhibition of renal arachidonic acid omega-hydroxylase activity. *American journal of physiology Regulatory, integrative and comparative physiology*. 2002; 283(3):R710–720.
50. Alonso-Galicia M, Drummond HA, Reddy KK, Falck JR, Roman RJ. Inhibition of 20-HETE production contributes to the vascular responses to nitric oxide. *Hypertension*. 1997; 29(1 Pt 2):320–325. [PubMed: 9039122]
51. Williams JM, Murphy S, Burke M, Roman RJ. 20-hydroxyeicosatetraenoic acid: a new target for the treatment of hypertension. *Journal of cardiovascular pharmacology*. 2010; 56(4):336–344. [PubMed: 20930591]
52. Dunn KM, Renic M, Flasch AK, Harder DR, Falck J, Roman RJ. Elevated production of 20-HETE in the cerebral vasculature contributes to severity of ischemic stroke and oxidative stress in spontaneously hypertensive rats. *American journal of physiology Heart and circulatory physiology*. 2008; 295(6):H2455–2465. [PubMed: 18952718]
53. Miyata N, Roman RJ. Role of 20-hydroxyeicosatetraenoic acid (20-HETE) in vascular system. *Journal of smooth muscle research = Nihon Heikatsukin Gakkai kikanishi*. 2005; 41(4):175–193. [PubMed: 16258232]
54. Alexanian A, Miller B, Roman RJ, Sorokin A. 20-HETE-producing Enzymes Are Up-regulated in Human Cancers. *Cancer genomics & proteomics*. 2012; 9(4):163–169. [PubMed: 22798501]
55. Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. *Prostaglandins & other lipid mediators*. 2011; 96(1–4):27–36. [PubMed: 21864702]
56. Simon LS. Role and regulation of cyclooxygenase-2 during inflammation. *The American journal of medicine*. 1999; 106(5B):37S–42S. [PubMed: 10390126]
57. Vanhoutte PM. COX-1 and vascular disease. *Clinical pharmacology and therapeutics*. 2009; 86(2):212–215. [PubMed: 19553934]
58. Feletou M, Huang Y, Vanhoutte PM. Endothelium-mediated control of vascular tone: COX-1 and COX-2 products. *British journal of pharmacology*. 2011; 164(3):894–912. [PubMed: 21323907]

59. Harder DR, Campbell WB, Roman RJ. Role of cytochrome P-450 enzymes and metabolites of arachidonic acid in the control of vascular tone. *Journal of vascular research*. 1995; 32(2):79–92. [PubMed: 7537544]
60. Harmon SD, Fang X, Kaduce TL, Hu S, Raj Gopal V, Falck JR, Spector AA. Oxygenation of omega-3 fatty acids by human cytochrome P450 4F3B: effect on 20-hydroxyeicosatetraenoic acid production. Prostaglandins, leukotrienes, and essential fatty acids. 2006; 75(3):169–177.
61. Tang Z, Salamanca-Pinzon SG, Wu ZL, Xiao Y, Guengerich FP. Human cytochrome P450 4F11: heterologous expression in bacteria, purification, and characterization of catalytic function. *Archives of biochemistry and biophysics*. 2010; 494(1):86–93. [PubMed: 19932081]
62. Alonso-Galicia M, Falck JR, Reddy KM, Roman RJ. 20-HETE agonists and antagonists in the renal circulation. *The American journal of physiology*. 1999; 277(5 Pt 2):F790–796. [PubMed: 10564244]
63. Cheng J, Ou JS, Singh H, Falck JR, Narsimhaswamy D, Pritchard KA Jr, Schwartzman ML. 20-hydroxyeicosatetraenoic acid causes endothelial dysfunction via eNOS uncoupling. *American journal of physiology. Heart and circulatory physiology*. 2008; 294(2):H1018–1026. [PubMed: 18156192]
64. Kroetz DL, Zeldin DC. Cytochrome P450 pathways of arachidonic acid metabolism. *Current opinion in lipidology*. 2002; 13(3):273–283. [PubMed: 12045397]
65. Sarkis A, Lopez B, Roman RJ. Role of 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acids in hypertension. *Current opinion in nephrology and hypertension*. 2004; 13(2):205–214. [PubMed: 15202615]
66. Sarkis A, Roman RJ. Role of cytochrome P450 metabolites of arachidonic acid in hypertension. *Current drug metabolism*. 2004; 5(3):245–256. [PubMed: 15180494]
67. Sun CW, Falck JR, Harder DR, Roman RJ. Role of tyrosine kinase and PKC in the vasoconstrictor response to 20-HETE in renal arterioles. *Hypertension*. 1999; 33(1 Pt 2):414–418. [PubMed: 9931139]
68. Gebremedhin D, Lange AR, Narayanan J, Aebly MR, Jacobs ER, Harder DR. Cat cerebral arterial smooth muscle cells express cytochrome P450 4A2 enzyme and produce the vasoconstrictor 20-HETE which enhances L-type Ca²⁺ current. *The Journal of physiology*. 1998; 507(Pt 3):771–781. [PubMed: 9508838]
69. Gebremedhin D, Lange AR, Lowry TF, Taheri MR, Birks EK, Hudetz AG, Narayanan J, Falck JR, Okamoto H, Roman RJ, Nithipatikom K, Campbell WB, Harder DR. Production of 20-HETE and its role in autoregulation of cerebral blood flow. *Circulation research*. 2000; 87(1):60–65. [PubMed: 10884373]
70. Harder DR, Gebremedhin D, Narayanan J, Jefcoat C, Falck JR, Campbell WB, Roman R. Formation and action of a P-450 4A metabolite of arachidonic acid in cat cerebral microvessels. *The American journal of physiology*. 1994; 266(5 Pt 2):H2098–2107. [PubMed: 8203608]
71. Gross ER, Nithipatikom K, Hsu AK, Peart JN, Falck JR, Campbell WB, Gross GJ. Cytochrome P450 omega-hydroxylase inhibition reduces infarct size during reperfusion via the sarcolemmal KATP channel. *Journal of molecular and cellular cardiology*. 2004; 37(6):1245–1249. [PubMed: 15572055]
72. Nithipatikom K, Gross ER, Endsley MP, Moore JM, Isbell MA, Falck JR, Campbell WB, Gross GJ. Inhibition of cytochrome P450 omega-hydroxylase: a novel endogenous cardioprotective pathway. *Circulation research*. 2004; 95(8):e65–71. [PubMed: 15388642]
73. Pat Kunert M, Drenjancevic I. 20-hydroxyeicosatetraenoic acid, endothelial dysfunction and hypertension. *Medicinski glasnik: official publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina*. 2011; 8(2):170–180.
74. Stec DE, Roman RJ, Flasch A, Rieder MJ. Functional polymorphism in human CYP4F2 decreases 20-HETE production. *Physiological genomics*. 2007; 30(1):74–81. [PubMed: 17341693]
75. Liu H, Zhao Y, Nie D, Shi J, Fu L, Li Y, Yu D, Lu J. Association of a functional cytochrome P450 4F2 haplotype with urinary 20-HETE and hypertension. *Journal of the American Society of Nephrology: JASN*. 2008; 19(4):714–721. [PubMed: 18235092]
76. Fava C, Montagnana M, Almgren P, Rosberg L, Lippi G, Hedblad B, Engstrom G, Berglund G, Minuz P, Melander O. The V433M variant of the CYP4F2 is associated with ischemic stroke in

- male Swedes beyond its effect on blood pressure. *Hypertension*. 2008; 52(2):373–380. [PubMed: 18574070]
77. Kaspera R, Totah RA. Epoxyeicosatrienoic acids: formation, metabolism and potential role in tissue physiology and pathophysiology. *Expert opinion on drug metabolism & toxicology*. 2009; 5(7):757–771. [PubMed: 19505190]
78. Renic M, Klaus JA, Omura T, Kawashima N, Onishi M, Miyata N, Koehler RC, Harder DR, Roman RJ. Effect of 20-HETE inhibition on infarct volume and cerebral blood flow after transient middle cerebral artery occlusion. *Journal of cerebral blood flow and metabolism: official journal of the International Society of Cerebral Blood Flow and Metabolism*. 2009; 29(3):629–639.
79. Chuang SS, Helvig C, Taimi M, Ramshaw HA, Collop AH, Amad M, White JA, Petkovich M, Jones G, Korczak B. CYP2U1, a novel human thymus-and brain-specific cytochrome P450, catalyzes omega- and (omega-1)-hydroxylation of fatty acids. *The Journal of biological chemistry*. 2004; 279(8):6305–6314. [PubMed: 14660610]
80. Chen P, Guo M, Wygle D, Edwards PA, Falck JR, Roman RJ, Scicli AG. Inhibitors of cytochrome P450 4A suppress angiogenic responses. *The American journal of pathology*. 2005; 166(2):615–624. [PubMed: 15681843]
81. Moreno JJ. New aspects of the role of hydroxyeicosatetraenoic acids in cell growth and cancer development. *Biochemical pharmacology*. 2009; 77(1):1–10. [PubMed: 18761324]
82. Guo M, Roman RJ, Falck JR, Edwards PA, Scicli AG. Human U251 glioma cell proliferation is suppressed by HET0016 [N-hydroxy-N'-(4-butyl-2-methylphenyl)formamidine], a selective inhibitor of CYP4A. *The Journal of pharmacology and experimental therapeutics*. 2005; 315(2):526–533. [PubMed: 16081682]
83. Guo M, Roman RJ, Fenstermacher JD, Brown SL, Falck JR, Arbab AS, Edwards PA, Scicli AG. 9L gliosarcoma cell proliferation and tumor growth in rats are suppressed by N-hydroxy-N'-(4-butyl-2-methylphenol) formamidine (HET0016): a selective inhibitor of CYP4A. *The Journal of pharmacology and experimental therapeutics*. 2006; 317(1):97–108. [PubMed: 16352703]
84. Alexanian A, Rufanova VA, Miller B, Flasch A, Roman RJ, Sorokin A. Down-regulation of 20-HETE synthesis and signaling inhibits renal adenocarcinoma cell proliferation and tumor growth. *Anticancer research*. 2009; 29(10):3819–3824. [PubMed: 19846914]
85. Kunau WH, Dommes V, Schulz H. beta-oxidation of fatty acids in mitochondria, peroxisomes, and bacteria: a century of continued progress. *Progress in lipid research*. 1995; 34(4):267–342. [PubMed: 8685242]
86. Wanders RJ, Vreken P, Ferdinandusse S, Jansen GA, Waterham HR, van Roermund CW, Van Grunsven EG. Peroxisomal fatty acid alpha- and beta-oxidation in humans: enzymeology, peroxisomal metabolite transporters and peroxisomal diseases. *Biochemical Society transactions*. 2001; 29(Pt 2):250–267. [PubMed: 11356164]
87. Wanders RJ, Komen J, Kemp S. Fatty acid omega-oxidation as a rescue pathway for fatty acid oxidation disorders in humans. *The FEBS journal*. 2011; 278(2):182–194. [PubMed: 21156023]
88. Sanders RJ, Ofman R, Dacremont G, Wanders RJ, Kemp S. Characterization of the human omega-oxidation pathway for omega-hydroxy-very-long-chain fatty acids. *The FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2008; 22(6):2064–2071.
89. Sanders RJ, Ofman R, Duran M, Kemp S, Wanders RJ. Omega-oxidation of very long-chain fatty acids in human liver microsomes. Implications for X-linked adrenoleukodystrophy. *The Journal of biological chemistry*. 2006; 281(19):13180–13187. [PubMed: 16547005]
90. Singh I, Khan M, Key L, Pai S. Lovastatin for X-linked adrenoleukodystrophy. *The New England journal of medicine*. 1998; 339(10):702–703. [PubMed: 9729143]
91. Singh I, Pahan K, Khan M. Lovastatin and sodium phenylacetate normalize the levels of very long chain fatty acids in skin fibroblasts of X- adrenoleukodystrophy. *FEBS letters*. 1998; 426(3):342–346. [PubMed: 9600263]
92. Engelen M, Ofman R, Dijkgraaf MG, Hijzen M, van der Wardt LA, van Geel BM, de Visser M, Wanders RJ, Poll-The BT, Kemp S. Lovastatin in X-linked adrenoleukodystrophy. *The New England journal of medicine*. 2010; 362(3):276–277. [PubMed: 20089986]

93. Verhoeven NM, Jakobs C. Human metabolism of phytanic acid and pristanic acid. *Progress in lipid research*. 2001; 40(6):453–466. [PubMed: 11591435]
94. Komen JC, Wanders RJ. Identification of the cytochrome P450 enzymes responsible for the omega-hydroxylation of phytanic acid. *FEBS letters*. 2006; 580(16):3794–3798. [PubMed: 16782090]
95. Wanders RJ, Komen JC. Peroxisomes, Refsum's disease and the alpha- and omega-oxidation of phytanic acid. *Biochemical Society transactions*. 2007; 35(Pt 5):865–869. [PubMed: 17956234]

PARTIAL I-HELIX
SEQUENCE (conserved xxGxxT
MOTIF)

Enzyme	
CYP4A11	DTFMF E GHDTTAS
CYP4B1	DTFMF E GHDTTTS
CYP4F2	DTFMF E GHDTTAS
CYP4F3	DTFMF E GHDTTAS
CYP4F8	DTFMF G GHDTTAS
CYP4F11	DTFMF E GHDTTAS
CYP4F12	DTFMF G GHDTTAS
CYP4F22	DTFMF E GHDTTSS
CYP4V2	DTFMF E GHDTTAA
CYP4X1	STFLL A GHDTLAA
CYP4Z1	KTFMF A GHDTTSS
CYP3A4	IIFIF A GYETTSS
CYP1A2	NDIFG A GFDTVTT
CYP2C9	VDLFG A GTETTST

Figure 1.

The CYP4 enzyme partial I-helix sequence compared to CYP3A4, CYP1A2, and CYP2C9. The conserved glutamic acid residue that forms a covalent bond with the heme cofactor in several CYP4 enzymes is highlighted.

Figure 2.
The CYP4F gene cluster on chromosome 19

Figure 3.
Fatty acid degradation pathways

Table 1

Summary of CYP4 inducers

Enzyme	Cell System	Inducer	Regulator(s) and/or nuclear receptor(s)	Reference
CYP4F2	HepG2, primary HH	lovastatin, mevastatin	SREBP	[26]
CYP4F2	HepG2	genistein, AICAR, resveratrol	AMPK	[28]
CYP4F3B	HepaRG	PGA1	unknown	[30]
CYP4F11	HaCaT	LG268 (aka LG100268)	RXR	[33]
CYP4F11	HaCaT	TNF α , IL-1B	JNK, AP-1 (RXR, RAR)	[33]
CYP4F11	HepG2	TNF α , TNF α +IMD0354	JNK, AP-1 (RXR, RAR) (+ NF- κ B)	[34]
CYP4A11	HepG2, primary HH	Fibrates	PPAR α	[11, 14]
CYP4Z1	T47-D (breast tumor)	dexamethasone, progesterone	PXR	[40]
CYP4Z1	MCF-7 (breast cancer)	dexamethasone	PXR	[40]
CYP4X1	HepG2	WY17643	PPAR α	[40]
CYP4F12	Primary HH	rifampicin	PXR	[35]

Table 2

Summary of CYP4 inhibitors.

Inhibitor	Structure	IC ₅₀ for 20-HETE formation in renal microsomes	Type of Inhibitor	Reference
Terminal acetylenic fatty acids (17- ODYA)		>5 μ M	mechanism-based	[48]
Terminal di-bromo fatty acids (DBDD)		2 μ M	reversible, competitive	[41]
Sulfonated fatty acids (10-SUYS) (DDMS)		10 μ M 2 μ M	mechanism-based reversible, competitive	[49, 50]
HET0016		35 nM	unknown	[42, 43]
TS-011		8 nM	unknown	[47]