Cytochrome P450 4F subfamily: At the crossroads of eicosanoid and drug metabolism

Auinash Kalsotra, Henry W. Strobel

Abstract

The cytochrome P450 4F (CYP4F) subfamily has over the last few years come to be recognized for its dual role in modulating the concentrations of eicosanoids during inflammation as well as in the metabolism of clinically significant drugs. The first CYP4F was identified because it catalyzed the hydroxylation of leukotriene B4 (LTB4) and since then many additional members of this subfamily have been documented for their distinct catalytic roles and functional significance. Recent evidence emerging in relation to the temporal change of CYP4F expression in response to injury and infection supports an important function for these isozymes in curtailing inflammation. Their tissue-dependent expression, isoform-based catalytic competence and unique response to the external stimuli imply a critical role for them to regulate organ-specific functions. From this standpoint variations in relative CYP4F levels in humans may have direct influence on the metabolic outcome through their ability to generate and/or degrade bioactive eicosanoids or therapeutic agents. This review covers the enzymatic characteristics and regulatory properties of human and rodent CYP4F isoforms and their physiological relevance to major pathways in eicosanoid and drug metabolism.

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Keywords: Cytochrome P450 4F; Inflammation; Leukotriene B4; Drug metabolism; Cytokines; Gene regulation

Abbreviations: 9-cisRA, 9-cis retinoic acid; AA, arachidonic acid; ATRA, all-trans retinoic acid; BaSO4, barium sulfate; C/EBP, CAAT enhancer binding protein; COX, cyclooxygenase; CYP, cytochrome P450; EET, epoxyeicosatrienoic acid; HETE, hydroxyeicosatetraenoic acid; IL, interleukin; LOX, lipoxygenase; LPS, lipopolysaccharide; LTB4, leukotriene B4; NF-κB, nuclear factor-κB; PMNL, polymorphonuclear leukocyte or neutrophil; PPAR, peroxisome proliferator activated receptor; RAR, retinoid A receptor; RXR, retinoid X receptor; TBI, traumatic brain injury; TNF-α, tumor necrosis factor-α.

Contents

1. Introduction ........................................... 590
2. History and discovery of cytochrome P450 4F (CYP4F) subfamily ........................................... 590
   2.1. Role of prostaglandins and leukotrienes in inflammation ........................................... 591
   2.2. Cytochrome P450 4F as prostaglandin and leukotriene hydroxylases ........................................... 592
   2.3. Cytochrome P450 4F involvement in 20-hydroxyeicosatetraenoic acid generation ........................................... 593
   2.4. Other endogenous substrates for cytochrome P450 4F ........................................... 593
3. Isoform- and tissue-specific expression of cytochrome P450 4F subfamily ........................................... 595
   3.1. Hepatic and extra-hepatic expression ........................................... 595
   3.1.1. Human cytochrome P450 4F ........................................... 595
   3.1.2. Rodent cytochrome P450 4F ........................................... 595
   3.2. Renal localization and cytochrome P450 4F expression in spontaneously hypertensive rats ........................................... 597
4. Genomic characterization and cytochrome P450 4F gene expression ........................................... 597
   4.1. Basal expression and inducibility ........................................... 598
1. Introduction

Cytochrome P450s (CYP), phase I drug metabolizing enzymes, comprise a superfamily of heme-thiolate proteins that play critical roles in endogenous as well as xenobiotic metabolism (Guengerich, 1991; Oritz de Montellano, 1995; Anzenbacher & Anzenbacherova, 2001; Nebert & Russell, 2002). CYP-dependent monooxygenases are found predominantly in the microsomal fraction of tissues, but such activities can also be seen in the mitochondria (Anandatheerthavarada et al., 1997, 1999; Robin et al., 2002). The microsomal CYP monooxygenase enzyme system, which is our interest here, has 3 major components: (a) the heme protein, CYP, (b) the flavoprotein, NADPH CYP-reductase and (c) phospholipids (Strobel et al., 1970; Coon, 2002, 2005). The reductase transfers 2 electrons to the CYP supplying reducing equivalents to the heme iron for reduction of molecular oxygen and product formation during catalysis (Strobel et al., 1995a).

Although first identified in liver, the presence and activity of CYP have been extended to extrahepatic tissues including kidney, colon, lung and brain (Guengerich & Mason, 1979; Strobel et al., 1997, 2001; Ding & Kaminsky, 2003; Zhao & Imig, 2003; Kroetz & Xu, 2004). Currently, >700 CYP have been characterized, inclusive of the many different species of organisms that have been studied (Nelson, 1999). These proteins are conveniently arranged into families and subfamilies on the basis of percent amino acid sequence identity (Nebert & Gonzalez, 1987). The individual CYP are represented by an Arabic number denoting the family, followed by an alphabetical letter designating the sub-family and an Arabic numeral representing the individual gene within the subfamily (Nelson et al., 1993; Nelson, 1999). This nomenclature is followed throughout this review.

Apart from having multiple forms, a fascinating fact about CYP is their broad substrate spectrum. While some CYP isoforms are fairly specific in their choice of substrate, such as those involved in steroidogenesis, the rest of them catalyze a large number of reactions.

Beyond their toxicological and pharmacological relevance, CYP enzymes play a central role in metabolizing endogenous products such as cholesterol, steroids, vitamins, eicosanoids and fatty acids (Capdevila et al., 1982; Andersson et al., 1989; McGiff, 1991; Kikuta et al., 1993; Kawashima et al., 1997; Lund et al., 1999; Node et al., 1999; Bylund et al., 2000; Capdevila et al., 2000; Thompson et al., 2000; Oliw et al., 2001; Nebert & Russell, 2002; Sontag & Parker, 2002; Cheng et al., 2004). Of particular interest is their role in the arachidonic acid (AA) cascade. AA, a polyunsaturated long-chain fatty acid esterified to cellular phospholipids, can be metabolized into various active and inactive products based on their cellular and physiological context. Prostaglandins (PG) and leukotrienes (LT) are the most widely studied metabolites of AA; however, several CYP in the subfamilies 2 and 4 catalyze conversion of AA into 20-hydroxyeicosatetraenoic acid (HETE) and epoxycosatrienoic acids (EET) (Fisslthaler et al., 1999; Capdevila et al., 1999; Capdevila et al., 2000; Lasker et al., 2000; Zeldin, 2001; Chuang et al., 2004; Kroetz & Xu, 2004). The focus of this review is the CYP4F subfamily which plays a pivotal role in metabolism of AA, its derivatives and many clinically important drugs.

2. History and discovery of cytochrome P450 4F (CYP4F) subfamily

CYP4F belong to a relatively newer CYP subfamily and were first discovered as part of an effort to define an inactivation pathway for leukotriene B_{4} (LTB_{4}), a powerful mediator of inflammation. In the early 1980s, Hansson et al. (1981) were the first to report that in a 2-step breakdown process, LTB_{4} is first ω-hydroxylated and then oxidized to a carboxylic acid in human leukocytes. Experiments with \textsuperscript{18}O demonstrated that the ω-hydroxyl group came from molecular oxygen suggesting the involvement of a mixed function oxidase in catalyzing this reaction. Subsequently, Shak and Goldstein (1985) reported that the ω-hydroxylase activity in the polymorphonuclear leukocyte (PMNL) resides in the microsomal fraction but the requirement of molecular oxygen and NADPH, as well as the inhibition by carbon monoxide and antibodies against NADPH-CYP reductase, confirmed that the activity is associated with a specific CYP (Shak & Goldstein, 1984, 1985; Soberman et al., 1985; Sumimoto et al., 1988). Soberman et al. (1987) later compared the activity of LTB_{4}-specific CYP against other isomers and analogues of LTB_{4} and found that both the triene bond
configuration and chirality of 5(S) and 12(R) hydroxy groups was preferred by this enzyme. But only after the advent of molecular biology and cDNA cloning techniques it became possible to clone the genes that encoded the LTB4 ω-hydroxylase. Chen and Hardwick (1993) and Kikuta et al. (1993) isolated simultaneously the cDNAs for the rat and human CYP with specific activity for LTB4 ω-hydroxylation, leading to the discovery of a new subfamily named CYP4F. Since then, the CYP4F subfamily has expanded to comprise 15 genes in human, rat and mouse (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Isozymes</th>
<th>Species</th>
<th>Substrates</th>
<th>Inducers</th>
<th>Repressors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP4F1</td>
<td>Rat</td>
<td>LTB4, AA</td>
<td>2-acetylaminofluorene, IL-1β, -6, TNF-α</td>
<td>Clofibrate, LPS, IL-10</td>
</tr>
<tr>
<td>CYP4F2</td>
<td>Human</td>
<td>LTB4, AA, Lipoxins, Vitamin E, Eicosatetraenoic acids, HETE</td>
<td>Retinoic acid</td>
<td>Clofibrate</td>
</tr>
<tr>
<td>CYP4F3</td>
<td>Rat</td>
<td>LTB4, AA, Lipoxins, Eicosatetraenoic acids, HETE</td>
<td>Retinoic acid, 12-myristate 13-acetate</td>
<td>-NK-</td>
</tr>
<tr>
<td>CYP4F4</td>
<td>Rat</td>
<td>LTB4, AA, Chloropromazine</td>
<td>IL-6, TNF-α</td>
<td>Clofibrate, LPS, IL-1β, -10</td>
</tr>
<tr>
<td>CYP4F5</td>
<td>Rat</td>
<td>LTB4, Chloropromazine</td>
<td>IL-1β, -6 and TNF-α</td>
<td>LPS, IL-10</td>
</tr>
<tr>
<td>CYP4F6</td>
<td>Rat</td>
<td>LTB4, Imipramine</td>
<td>BaSO4</td>
<td>LPS, IL-10</td>
</tr>
<tr>
<td>CYP4F8</td>
<td>Human</td>
<td>AA, Prostaglandin H2 analogues, Docosahexaenoic acid, Docosapentaenoic acid</td>
<td>-NK-</td>
<td>-NK-</td>
</tr>
<tr>
<td>CYP4F11</td>
<td>Human</td>
<td>Erythromycin, Chloropromazine, Theophylline, Benzphetamine, Ethylmorphine</td>
<td>-NK-</td>
<td>-NK-</td>
</tr>
<tr>
<td>CYP4F12</td>
<td>Human</td>
<td>Ebatine, AA, Prostaglandin H2 analogues, Docosahexaenoic acid, Docosapentaenoic acid</td>
<td>-NK-</td>
<td>-NK-</td>
</tr>
<tr>
<td>CYP4F13</td>
<td>Mouse</td>
<td>-NK-</td>
<td>-NK-</td>
<td>-NK-</td>
</tr>
<tr>
<td>CYP4F14</td>
<td>Mouse</td>
<td>LTB4, Lipoxins, Hydroxyeicosatetraenoic acids</td>
<td>-NK-</td>
<td>Clofibrate, C. rodentium</td>
</tr>
<tr>
<td>CYP4F15</td>
<td>Mouse</td>
<td>-NK-</td>
<td>Clofibrate</td>
<td>LPS, C. rodentium</td>
</tr>
<tr>
<td>CYP4F16</td>
<td>Mouse</td>
<td>-NK-</td>
<td>C. rodentium</td>
<td>Clofibrate</td>
</tr>
<tr>
<td>CYP4F18</td>
<td>Mouse</td>
<td>LTB4</td>
<td>LPS, C. rodentium</td>
<td>-NK-</td>
</tr>
</tbody>
</table>

-NK-: not known.

In contrast to PG, LT are made predominantly by inflammatory cells like PMNL, macrophages, and mast cells (Funk, 2001). Two enzymes, 5-lipoxygenase (LOX) (Funk et al., 1987) and leukotriene A4 (LTA4) hydrolase (Minami et al., 1987), are required for LT biosynthesis from AA (Fig. 1). 5-LOX-activating protein is also required for 5-LOX activity (Dixon et al., 1990), but its molecular mechanism remains elusive. Although 5-LOX was initially purified as a cytosolic protein, the enzyme is known to translocate to the nuclear envelope (Brock et al., 1995; Flamand et al., 2002). Therefore, it is now widely accepted that the nuclear membrane is a major site of LT production. 5-LOX catalyzes AA to form LTA4. LTA4 undergoes transformation to 3 possible fates depending on the cellular context; hydrolysis, conjugation with glutathione, or

![Fig. 1. Enzymatic pathway for formation and metabolism of LTB4.](https://example.com/f1.png)
transcellular metabolism to generate bioactive eicosanoids (Gronert et al., 1999). Hydrolytic attack of LTA\textsubscript{4} by LTA\textsubscript{4} hydrolase in the cytoplasm, and potentially in the nucleus, yields LTB\textsubscript{4}, a potent neutrophil chemota\textsubscript{c}ctant and stimulator of leukocyte adhesion to endothelial cells (Samuelsson, 1983; Serhan et al., 1996; Peters-Golden & Brock, 2001). LTA\textsubscript{4} conjugation with glutathione to form LTC\textsubscript{4} at the nuclear envelope is catalyzed by LTC\textsubscript{4} synthase (Funk, 2001). LTC\textsubscript{4} is transported out of the cell by transporters such as the multidrug resistance-associated protein and the peptide moiety of LTC\textsubscript{4} is subjected to extracellular metabolism, forming LTA\textsubscript{4} and LTE\textsubscript{4}. These 3 LT comprise the cysteinyl LT, sometimes also referred as “slow-reacting substance of anaphylaxis” for their slow and sustained smooth muscle contracting abilities (Samuelsson, 1983). The biological properties of cysteiny\textsubscript{l} LT are well studied and their discussion is beyond the scope of this review.

LTB\textsubscript{4}, a potent eicosanoid lipid mediator, carries out a range of homeostatic and pathological functions (Samuelsson, 1983; Samuelsson et al., 1987). Numerous studies have shown elevated tissue or fluid levels of LTB\textsubscript{4} to be associated with the pathogenesis of several inflammatory diseases (Jala & Haribabu, 2004) such as asthma (Barnes et al., 1998; Luster & Tager, 2004; Terawaki et al., 2005), autoimmune dermatitis (Koro et al., 1999), rheumatoid arthritis (Griffiths et al., 1995), inflammatory bowel disease (Ikehata et al., 1995), hepatic cirrhosis (Claria et al., 1998), fibrosis, and cholestasis (Keppler et al., 1985). Furthermore, elevated LTB\textsubscript{4} levels have been implicated during injury and trauma (Denzlinger et al., 1985; Xu et al., 1990; Tarlowe et al., 2003).

When studied in vitro, LTB\textsubscript{4} promotes chemotaxis of PMNL, monocytes and fibroblasts indicating that it is a key component of the inflammatory immune response (Ford-Hutchinson et al., 1980; Martin et al., 1989; Borget & Naccache, 1990; Bailie et al., 1996). It also causes PMNL degranulation (Naccache et al., 1979) and acts as a calcium ionophore, promoting calcium movement into cells (Naccache et al., 1979; Yokomizo et al., 2000). The actions of LTB\textsubscript{4} are exerted via BLT (Yokomizo et al., 1997), cell-surface GPCR isolated from HL-60 cells (Kamohara et al., 2000; Yokomizo et al., 2000). More recently, the LTB\textsubscript{4}-BLT pathway was linked to immune system activation and early effector T cell recruitment to the inflamed tissues (Goodarzi et al., 2003; Ott et al., 2003; Tager et al., 2003). Two lines of BLT1-null mice have been generated (Haribabu et al., 2000; Tager et al., 2000). Neutrophils and macrophages of these mice lack LTB\textsubscript{4} induced calcium mobilization and exhibit reduced accumulation of eosinophils, neutrophils and macrophages.

2.2. Cytochrome P450 4F as prostaglandin and leukotriene hydroxylases

Both PG and LT are short lived molecules produced as well as metabolized at the target tissue. PG are metabolized into compounds detected in the urine with 14, 16, or 18 carbon atoms via many steps including oxidation of 15-

\text{hydroxy} group, reduction of C\textsubscript{13}–C\textsubscript{14} double bond, \text{β-oxidation} and \text{ω-oxidation}. In 1978, Powell and Solomon reported that microsomes from lung and liver of pregnant rabbits are highly active in the \text{ω-oxidation} of PG, their metabolites and some PG\textsubscript{2α} analogues (Powell & Solomon, 1978). Furthermore, the PG\textsubscript{2α} \text{ω-oxidation} activity of lung microsomes was shown to increase during pregnancy. Later, the enzymes responsible for carrying out PG metabolism were characterized and shown to be CYP to be members of CYP4A subfamily.

Masters’s laboratory along with Kuusnose’s group were pioneers in characterizing the PG \text{ω-oxidation} activities of CYP4A isoforms (Okita et al., 1981; Kaku et al., 1984; Kusunose et al., 1989; Muerhoff et al., 1989; Yokotani et al., 1989; Johnson et al., 1990; Kikuta et al., 1990; Masters et al., 1993; Roman et al., 1993). Subsequently, CYP4A were also shown to be highly active in metabolizing short and medium-chain fatty acids. Interestingly, as a part of this investigation, AA was found to be an efficient substrate for CYP4A. These findings suggested that CYP4A had dual physiological functions; metabolic inactivation of PG and regulation of fatty acid levels. Today, however, the CYP4F subfamily has also gained similar recognition in PG and AA metabolism.

LTB\textsubscript{4}, like other eicosanoids, is rapidly metabolized in vivo by multiple pathways into biologically inactive products. One of the metabolic pathways proceeds via \text{ω-oxidation} of LTB\textsubscript{4} to form 20-

\text{hydroxy-LTB4} which is characteristic of the CYP4F subfamily (Kikuta et al., 2002) (Fig. 1). \text{β-oxidation} is known to be a predominant pathway of LTB\textsubscript{4} metabolism in the hepatocyte after the initial \text{ω-oxidation} of the methyl terminus and alcohol dehydrogenase-mediated formation of an \text{ω-carboxyl} moieties (Shirley & Murphy, 1990). Several other enzymatic pathways have been discovered that transform LTB\textsubscript{4} into inactive products, including the 12-

\text{hydroxyeicosanoid} dehydrogenase (Yokomizo et al., 1993) and 10,11-

\text{reductase pathways} (Wainwright & Powell, 1991), which ultimately convert the conjugated triene into a conjugated diene moiety.

Despite several inactivation possibilities, \text{ω-oxidation} by CYP4F was shown to be the major degradative pathway for this eicosanoid (Shak & Goldstein, 1984; Shak et al., 1985; Harper et al., 1986; Yokomizo et al., 2001; Kikuta et al., 2002; Berry et al., 2003). Since the initial discovery of neutrophil-specific CYP4F3 by Kikuta et al., several new members in this subfamily have been discovered. To date, 5 human CYP4F genes with 2 additional splice variants have been identified (Kikuta et al., 1993, 1994; Christmas et al., 1999; Kikuta et al., 1999b; Bylund et al., 2000; Cui et al., 2000; Bylund et al., 2001; Hashizume et al., 2001a). In addition, rats have 4 and mice have 5 CYP4F related genes (Chen & Hardwick, 1993; Kawashima & Strobel, 1995; Kikuta et al., 2000a; Cui et al., 2001; Kikuta et al., 2002) (Table 1).

Metabolism of LTB\textsubscript{4} in human PMNL by CYP4F3 to the 20-

\text{hydroxy} derivative leads to a dramatic loss of both its chemotactic and aggregation activity on PMNL (Powell, 1984; Kikuta et al., 1993). Also, the 20-

\text{hydroxy} compound is much less active in degranulation of human leukocytes, a result also been seen in rat leukocytes and rabbit lung strips (Samuelsson et al., 1987). Further, 20-OH LTB\textsubscript{4} is very unstable and is readily metabolized to 20-

COOH LTB\textsubscript{4} (Yokomizo et al., 2001). Inspite of a common feature that all CYP4F are capable of...
LTB4 hydroxylation, there is ample evidence for considerable differences in their substrate specificities (e.g., \( K_{\text{m}} \) of CYP4F2 for LTB4 [60 \( \mu \)M] is 94-fold higher than CYP4F3 [0.64 \( \mu \)M]), as well as, their isofrom- and tissue-specific expression (Kawashima et al., 1997; Kikuta et al., 1998b; Bylund et al., 2000; Lasker et al., 2000; Bylund et al., 2001; Christmas et al., 2001; Kalsotra et al., 2002; Kikuta et al., 2002).

CYP4F8 oxygenates AA to 18(\( \Delta \))-HETE and hydroxylates 2 PG endoperoxides, PGG1 and PGG2 to their 19-hydroxy-derivatives (Bylund et al., 2000). The biological significance of these metabolites is not clear at present. Three stable PGG2 analogs; 9,11-epoxymethano-PGG2 (U-44069), 11,9-epoxy-methano-PGH2 (U-46619), and 9,11-diazo-15-deoxy-PGH2 (U-51605) are also rapidly converted to their 18- and 19-hydroxy-products (Bylund et al., 2000). On the other hand, little or no activity is evident with LTB4, PGD2, PGE1, PGE2 or PGF2\( \alpha \). Recombinant CYP4F11 performs minimal catalysis of eicosanoids such as LTB4, PG or AA (Kalsotra et al., 2004).

CYP4F12 on the other hand shares its catalytic properties with CYP4F8. Recombinant CYP4F12 also metabolizes PGG2 analogs by \( \omega-2 \) and \( \omega-3 \) and AA by \( \omega-3 \) hydroxylation. Additionally CYP4F8 and 4F12 both catalyze epoxidation of docosahexaenoic acid and docosapentaenoic acid (Stark et al., 2005). CYP4F12 enzymes with \( \omega-3 \)-hydroxylase activity contain a heme-binding glutamate residue, whereas CYP4F8 and CYP4F12 with \( \omega-2 \) and \( \omega-3 \) hydroxylase activities have a glycine residue in this position. Stark et al. (2005) showed that the mutant CYP4F8 Gly328Glu oxidized U-51605 and U-44069 as recombinant CYP4F8, but the hydroxylation of AA was shifted from C18 to C19. Thus single amino acid substitutions in the mutant CYP4F8 Gly328Glu oxidized U-51605 and U-44069 or no activity is evident with LTB4, PGD2, PGE1, PGE2 or PGF2\( \alpha \). Recombinant CYP4F11 oxidizes U-51605 and U-44069 and 4f18 is known. Recombinant Cyp4f14 catalyzes \( \omega \)-hydroxylation of LTB4 and PAG1, with no detectable activity against arachidonate (Kikuta et al., 2000a). Cyp4f18 on the other hand prefers to hydroxylate LTB4 at \( \omega-1 \) and \( \omega-2 \) positions (Christmas et al., 2005). Catalytic functions of other recently cloned mouse Cyp4f isoforms—4f13, 4f15 and 4f16 remain to be characterized.

2.3. Cytochrome P450 4F involvement in 20-hydroxyeicosatetraenoic acid generation

20-HETE is an \( \omega \)-hydroxylated derivative and a major metabolite of AA produced in the kidney of several species, including humans (Schwartzman et al., 1990). Significant progress has been made in understanding the biological function of 20-HETE and the molecular mechanisms that determine the intracellular levels of this eicosanoid. It is known that 20-HETE regulates renal vascular tone (Imig et al., 1996; Zou et al., 1996), inhibits Na\(^+\)/K\(^-\) ATPase (Schwartzman et al., 1985) and the 70-ps K\(^+\) channel in the medullary thick ascending limb (Escalante et al., 1991), auto-regulates renal blood flow and glomerular filtration rate (Zou et al., 1994) and causes dose-dependent vasoconstriction of renal arcuate arteries (Ma et al., 1993).

20-HETE is primarily produced by the members of CYP4 family. The CYP4A subfamily was the first to be characterized to catalyze AA \( \omega \)-hydroxylation but recently the CYP4F subfamily has been shown to be equally important in AA metabolism (Kroetz & Xu, 2004). CYP4A11 is the major human CYP4A isofrom and CYP4A11 purified from both liver and kidney is able to carry out both \( \omega \) and \( \omega-1 \) hydroxylation of AA (Powell et al., 1998; Lasker et al., 2000). However, in both studies, CYP4F2-catalyzed 20-HETE formation was shown to be quantitatively more important than CYP4A11. CYP4F3B, a liver-specific splice variant of CYP4F3 gene, also metabolizes AA to form 20-HETE (Christmas et al., 2001).

In rats, CYP4A1 is the most active 4A isofrom to metabolize AA followed by CYP4A2/4A3 and 4A8 with respective \( K_{\text{cat}} \) values of 6, 2 and 1 min\(^{-1} \) (Nguyen et al., 1999; Hoch et al., 2000). Recently, Xu et al. (2004), conducted a direct comparison of the 4 rat CYP4F isoforms for their ability to metabolize AA. CYP4F1 and 4F4 were identified as major catalysts for 20-HETE formation and the \( K_{\text{cat}} \) values for CYP4F1 (9 min\(^{-1} \) and 4F4 (11 min\(^{-1} \)) were found to be quite similar to what was reported for CYP4A isoforms (Hoch et al., 2000). Similar competence towards AA implies a redundant function for CYP4A and 4F enzymes in 20-HETE formation; however, distinct patterns and levels of expression observed between the various members suggest unique roles for the individual isoforms.

2.4. Other endogenous substrates for cytochrome P450 4F

Apart from AA, LTB4 and PG, CYP4F also metabolize LX, EET and HETE (Kikuta et al., 1994; Kawashima et al., 1997; Kikuta et al., 1998b; Bylund et al., 1999b; Kikuta et al., 1999a; Bylund et al., 2000, 2003; Xu et al., 2004). The chemical structures of the CYP4F substrates are shown in Fig. 2. A list of individual CYP4F isoforms and their substrates is also shown in Table 1. EET, DiHETE, and 19- and 20-HETE act predominantly as autocrine factors and are capable of altering the vascular tone in many tissues (Roman, 2002). While LT and PG amplify the inflammatory response, LX on the other hand are potent mediators in resolving inflammation (Serhan, 1997). Boucher et al. (1991) were the first to report that rat liver...
microsomes hydroxylate LXA₄ and LXB₄ at the ω or ω-1 position. Kikuta et al. (1999a) demonstrated that although LXA₄ is ω-hydroxylated 3.2-fold more rapidly than LTB₄ by the purified CYP4F1, it has no activity against LXB₄.

Likewise, CYP4F3 catalyzes hydroxylation of various eicosanoids such as, LXA₄, LXB₄, 5-HETE and 12-HETE, and 12-OH-stearate although $K_m$ values for these substrates are much higher than that for LTB₄ (Kikuta et al., 1998b). In contrast, little activity of CYP4F3 is detected toward laurate, palmitate, arachidonate, 15-HETE, and PGE₁, all of which serve as excellent substrates for the CYP4A subfamily (Kikuta et al., 1998b, 2002). The findings that CYP4F3 has a broad substrate specificity are in agreement with the previous observations that human neutrophils metabolize other eicosanoids such as 5-HETE (O’Flaherty et al., 1986), 12-HETE (Wong et al., 1984) and LX (Sumimoto et al., 1993). Similar to CYP4F3, purified 4F2 also catalyzes ω-hydroxylation of various eicosanoids and related compounds including LXA₄, 8-HETE, and 12-HETE. Interestingly, murine CYP4F orthologues also show high degree of 8-HETE catalysis (Kikuta et al., 1999a, 2000b). 8(S)-HETE is generated from AA by 8(S)-LOX, which was reported to be located in the mouse skin and inducible with treatment of phorbol esters (Jisaka et al., 1997). Investigation of CYP4F presence in skin will shed more light on the significance of these catabolic pathways. The expression of multiple CYP families in the skin (Du et al., 2005) and their role in synthesis and degradation of bioactive lipids is being actively carried out (Du et al., 2004, 2006).

AA is also hydroxylated by multiple CYP to form regio-selective-EET and their corresponding DiHETE (Fang et al., 2001). EET are synthesized by P450 epoxygenase (Zeldin et al., 1996; Zeldin, 2001) and are endowed with important vasodilating and anti-inflammatory properties (Kroetz & Zeldin, 2002; Gross et al., 2005; Spiecker & Liao, 2005). ω-hydroxylation of these derivatives is considered to be associated with detoxification; however, a recent report by Cowart et al. (2002) has challenged the notion of EET ω-hydroxylation as a degradative pathway. Instead the authors provide evidence for the involvement of this process in the biogenesis of secondary messengers capable of binding to the nuclear receptor, peroxisome proliferator activated receptor (PPAR) α, with high affinity (Cowart et al., 2002).

In an attempt to pinpoint exactly the human isoforms responsible for the regio-selective oxidation of fatty acid epoxides, Le Quéré et al. investigated the hydroxylation of 3 epoxidized derivatives from the C₁₈ family. Human hepatic microsomes were able to convert them to ω-hydroxy-C₁₈-epoxides with apparent $K_m$ of between 27.6 and 175 μM (Le Quere et al., 2004). Among different recombinant CYP tested, CYP4F2, 4F3A and 4F3B robustly catalyzed the ω-hydroxylation of C₁₈-epoxides (Le Quere et al., 2004). While CYP4F2 and 4F3 exhibited preferences for ω-hydroxylation of Z 8(9)-EET, human liver microsomes preferred Z 11(12)-EET and, to a lesser extent, Z 8(9)-EET. In addition the vicinal diol from both C₁₈-epoxides and EET was ω-hydroxylated by liver microsomes and by CYP4F2 and 4F3 (Le Quere et al., 2004). The formation of this novel class of endogenous polyoxidized fatty

![Fig. 2. Chemical structures of eicosanoid metabolized by CYP4F subfamily. The chemical structures of the substrates were drawn using ISIS DRAW. The asterisks indicate the specific sites for metabolism. HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; 8,9-Dihydroxy-ET, 8,9-dihydroxyeicosatrienoic acid.](image-url)
acids is interesting in the light of the catalytic potential of CYP4F isoforms that are already known to inactivate various eicosanoids.

Recently Sontag and Parker (2002) described a pathway involving CYP4F2-mediated ω-hydroxylation of the tocopherol phytol side chain followed by stepwise removal of 2- or 3-carbon moieties, ultimately yielding the 3′-carboxyhercmonanol metabolite that is excreted in urine. Tocopherol-ω-hydroxylase exhibited similar binding affinities but markedly higher catalytic activities for gamma-tocopherol than alpha-tocopherol, suggesting a role for this pathway in the preferential physiological retention of alpha-tocopherol and elimination of gamma-tocopherol. Furthermore, sesamin was shown to potently inhibit tocopherol-omega-hydroxylase activity exhibited by CYP4F2 (Sontag & Parker, 2002). Since dietary sesamin results in elevated tocopherol levels in vivo (Yamashita et al., 1999; Kamal-Eldin et al., 2000; Cooney et al., 2001), this pathway is suggested to represent a functionally significant means of regulating vitamin E status (Parker et al., 2004; Sontag & Parker, 2004).

Taken together these observations strengthen the notion that CYP4F subfamily previously thought to be specific in LTB₄ catalysis has a much broader range of substrates.

3. Isoform- and tissue-specific expression of cytochrome P450 4F subfamily

Maximum expression of several CYP is detected in the liver, as it is the main site for endogenous substrates and drug metabolism. It is, however, important to study CYP in extrahepatic tissues, since they are known to play important roles in organ function as well as having pathophysiological states associated with them (Nebert & Russell, 2002). In the lung, heart and kidney, CYP4F presence is important since they are known to play important roles in organ function as well as having pathophysiological states associated with them (Nebert & Russell, 2002). In the lung, heart and kidney, CYP4F presence is important since P450-mediated AA metabolites are implicated in the regulation of blood pressure (Roman, 2002). The specific content of CYP in the brain is much lower than that in the liver and kidney but CYP play a unique and important role in the brain by taking part in neurosteroid, fatty acid as well as xenobiotic metabolism (Ravindranath et al., 1990, 1995; Kamal-Eldin et al., 2000; Cooney et al., 2001), this pathway is suggested to represent a functionally significant means of regulating vitamin E status (Parker et al., 2004; Sontag & Parker, 2004).

3.1. Hepatic and extra-hepatic expression

3.1.1. Human cytochrome P450 4F

The localization of CYP4F3 is restricted to myeloid cells, whereas CYP4F2 is distributed in liver, kidney, skin, several other tissues and tumors, but not in myeloid cells (Kikuta et al., 2005). CYP4F8 was cloned from seminal vesicles (Bylund et al., 2001; Hashizume et al., 2001a) from human liver and small intestine, respectively. Northern blot analysis showed 3 major transcripts of CYP4F12, which were detected in liver, kidney, colon, small intestine, respectively. Northern blot analysis showed 3 major transcripts of CYP4F12, which were detected in liver, kidney, colon, small intestine, respectively. Northern blot analysis showed 3 major transcripts of CYP4F12, which were detected in liver, kidney, colon, small intestine, respectively.

An anti-peptide polyclonal antibody against the C-terminal end of CYP4F8 screened 50 human tissues for 4F8 protein immunoreactivity. CYP4F8 protein was detected in seminal vesicles, epidermis, hair follicles, sweat glands, cornal epithelium, proximal renal tubules, and epithelial linings of the gut and urinary tract. A prominent induction of CYP4F8 immunoreactivity and mRNA in psoriasis in comparison with unaffected epidermis of the same patients was observed (Stark et al., 2003). The cDNA of CYP4F8 from plucked scalp hair roots was found to be identical to the genital cDNA sequence.

In addition to CYP4F2, 4F3 and 4F8, 2 additional CYP4F members, 4F11 and 4F12, were recently reported to be expressed in human liver. By a combination of cDNA library screening and rapid amplification of cDNA ends analysis, CYP4F11 was cloned from liver (Cui et al., 2000). CYP4F11 is expressed in human liver, followed by kidney, heart, brain and skeletal muscle (Cui et al., 2000). Inability to catalyze eicosanoid metabolism suggests a different endogenous role for CYP4F11 (Kalsotra et al., 2004). Alternatively, recombinant CYP4F11 is quite active in drug metabolism. CYP4F12 was cloned separately by 2 independent groups (Bylund et al., 2001; Hashizume et al., 2001a) from human liver and small intestine, respectively. Northern blot analysis showed 3 major transcripts of CYP4F12, which were detected in liver, kidney, colon, small intestine, respectively. Northern blot analysis showed 3 major transcripts of CYP4F12, which were detected in liver, kidney, colon, small intestine, respectively.
Cyp4f15, Cyp4f16, and Cyp4f18). CYP4F1 was originally isolated from a cDNA library of a 2-acetylamino fluorene-induced transplantable rat hepatic tumor (Chen & Hardwick, 1993). Although CYP4F1 is normally found in rat liver, its expression is much higher in various rat hepatomas. CYP4F4, 4F5 and 4F6 were originally isolated from rat brain (Kawashima & Strobel, 1995) but are found in other tissues such as kidney, lung, heart and spleen as well (Kalsotra et al., 2002). Subsequently, the mouse counterpart of CYP4F1, termed Cyp4f14, was identified (Kikuta et al., 2000a). Cyp4f14 expression has been detected in the liver, kidney and brain (Kikuta et al., 2000a; Stec et al., 2003). Cui et al. reported the molecular cloning of 2 additional mouse Cyp4F genes, 4f15 and 4f16. Both isoforms are expressed in liver and also in extrahepatic tissues but the patterns of expression are slightly different (Cui et al., 2001; Stec et al., 2003). Cyp4f15 mRNA is expressed in brain, kidney and lung to much lesser extents, whereas Cyp4f16 mRNA is detected in kidney, heart, brain, spleen, and lung (Cui et al., 2001). Cyp4f18 is the functional orthologue of CYP4F3A in mouse PMNL but is also highly expressed in ovary and dendritic cells (Christmas et al., 2005).

Careful analysis of CYP4F distribution and expression in different rat tissues provides important insights into their putative functions. Isoform specific distribution profile of rat CYP4F is shown in Fig. 3. CYP4F1 is highly expressed in the liver, kidneys and brain yet no 4F1 is detected in the lungs (Kalsotra et al., 2002). Kikuta et al. (1999a) demonstrated that purified CYP4F1 is quite active as an hydroxylase towards LTB4, LX, PG and HETE. High expression of CYP4F1 implies it may contribute heavily to the endogenous eicosanoid pool that affects the delicate balance of hepatic and renal physiological functions. CYP4F5, on the other hand, has a low basal expression (Fig. 3) in liver and kidney (Kalsotra et al., 2002, 2003a) but a high $V_{\text{max}}$ (30 nmol product/min/mg protein for CYP4F5 vs. 15 nmol product/min/mg protein for CYP4F1, 0.5 nmol product/min/mg protein for CYP4F4 and 1.1 nmol product/min/mg protein for CYP4F6) and a low $K_m$ for LTB4 (9.7 μM for CYP4F5 vs. 134 μM for CYP4F1, 45.5 μM for CYP4F4 and 26 μM for CYP4F6) (Kawashima et al., 1997; Kikuta et al., 1999a; Bylund et al., 2003). This indicates that CYP4F5 has a high kinetic competence for hydroxylation of LTB4. Furthermore, the lower basal levels but higher catalytic efficiency of CYP4F5 implies it to be important in regulating LTB4 metabolism during an inflammatory response.

To address whether CYP4F are active in rat tissues, isoform-based CYP4F expression profiles was compared to the published ω-side chain oxygenated LTB4 metabolite profile formed by the respective tissues and the purified recombinant CYP4F associated activity (Kawashima et al., 1997; Kikuta et al., 1999a; Bylund et al., 2003). This analysis indicates that 20-hydroxylation is the dominating oxygenation pathway for LTB4 in the liver and brain. The observation fits well with the high expression of CYP4F1 and 4F4 in these tissues (Fig. 3). Similarly, the presence of all CYP4F in the brain is in agreement with the formation of 18-, 19- and 20-hydroxy-LTB4 in this organ (Bylund et al., 2003). In the lung, where 18- and 19-hydroxylation is found to be the predominant pathway, CYP4F5 and 4F6 are the primarily expressed isoforms (Fig. 3).

While ω-oxidation is an LTB4 inactivation pathway (based on the fact that 20-hydroxy-LTB4 undergoes rapid conversion to 20-carboxy-LTB4 and subsequent β-oxidation; Shak & Goldstein, 1984; Wheelan et al., 1999; Kikuta et al., 2002), 18- and 19-hydroxy-LTB4 differ from 20-hydroxy-LTB4 in that respect. 19-Hydroxy-LTB4 has been shown to undergo dehydrogenation leading to 19-oxo-LTB4 in rat PMNL (Powell & Gravelle, 1990), and it is possible that 18-hydroxy-LTB4 can undergo 18-dehydrogenation also. However, it seems unlikely for 18- and 19-hydroxy-LTB4 to be structurally modified in a way that they could be subjected to β-oxidation. Whether ω-1 and ω-2 hydroxylated products also participate in LTB4 inactivation merits further studies.

CYP4F are not only expressed a tissue-dependent manner but also display sexual dimorphism in both rat and mouse (Kalsotra et al., 2002; Stec et al., 2003). CYP4F are enriched in female rats but different female tissues show different preferences in terms of CYP4F expression when compared to their male counterparts (Kalsotra et al., 2002). Ovariectomy produces a considerable decrease in CYP4F4 message in the liver and kidney. This loss of expression can be partially recovered with estrogen treatment in both tissues (Kalsotra et al., 2002). Stec et al. (2003) have reported sex differences in the renal expression pattern of mouse CYP4F isoforms. In the glomeruli, males expressed 4f13, 4f14, and 4f15 isoforms, whereas females expressed 4f13, 4f16, and 4f18 isoforms. Interestingly, female mice deficient in BLT1 receptor show resistance to platelet activating factor-induced anaphylaxis (Haribabu et al., 2000), suggesting sex-related involvement of BLT1 and possibly LTB4 in anaphylaxis. This raises the question whether higher CYP4F expression plays a protective role in females during acute and/or chronic inflammation.

Fig. 3. Isoform- and tissue-specific rat CYP4F distributions. mRNA levels for individual forms were assayed using real time quantitative PCR. The data in the modified figure is taken from Kalsotra et al. (2002, 2003a). The pie charts represent the relative distribution of CYP4F isoforms in (A) liver, (B) kidney, (C) lung and (D) brain.
3.2. Renal localization and cytochrome P450 4F expression in spontaneously hypertensive rats

A recent comparison of AA ω-oxidation revealed that CYP4F isoforms are as efficient as the CYP4A in catalyzing P450 4F expression in spontaneously hypertensive rats (Kroetz & Xu, 2004). The detection of AA ω-hydroxylase activity has been localized to the proximal tubules, whereas AA ω-1 hydroxylation is more widespread throughout the nephron (Omata et al., 1992b). Also, 20-HETE causes vasoconstriction of renal arteries and is therefore considered pro-hypertensive while its natriuretic and diuretic effects on renal tubules favor lowering of blood pressure (Roman, 2002; Zhao & Imig, 2003; Kroetz & Xu, 2004). Thus localization of CYP4F along the nephron to more discrete regions might prove useful in discerning the relative importance of this subfamily in kidney functions compared to CYP4A which are highly expressed in the S2 and S3 fragments of proximal tubules but are rare in the cortical collecting, distal convoluted and connecting tubules (Schwartzman et al., 1996). Targeted disruption of the cortical collecting, distal convoluted and connecting tubules S2 and S3 fragments of proximal tubules but are rare in the nephron (Omata et al., 1992b). Since CYP4F1 is one of the major eicosanoid formation and renal function in the young SHR. (Kroetz et al., 1997). Blood pressure becomes elevated in SHR compared to WKY rats from 5 weeks of age and remains elevated thereafter (Beierwaltes et al., 1982). Differences in AA ω-hydroxylation activity in renal microsomes from SHR and WKY rats have been known for several years (Imaoka & Funae, 1991; Omata et al., 1992a; Imig et al., 1993; Stec et al., 1996b); however, altered CYP4A expression in the SHR is not completely coincident with the increased blood pressure. Increased expression of CYP4A3 and A4A has been suggested to be critical to the early changes in the eicosanoid formation and renal function in the young SHR (Kroetz et al., 1997). Since CYP4F1 is one of the major catalysts for 20-HETE biosynthesis (Xu et al., 2004) and it is consistently upregulated in SHR compared to normotensive rats (Kalsotra et al., 2005), 4F1 is speculated to be an important enzyme in the development of hypertension in the SHR.

4. Genomic characterization and cytochrome P450 4F gene expression

The genomic structures of CYP4F genes have been solved either by traditional library screening or by computer analysis using bioinformatics research tools. The human CYP4F genes CYP4F2, CYP4F3, CYP4F8, CYP4F11, and CYP4F12 are located at chromosome 19p13 and compose a large gene cluster (Kikuta et al., 1998a; Christmas et al., 1999; Bylund et al., 2000; Cui et al., 2000; Bylund et al., 2001). Gene structures of these CYP4F enzymes are similar with each other and their splicing sites are almost identical (Kikuta et al., 2002). These results suggest that the human CYP4F subfamily may have increased numbers by gene duplication on chromosome 19, resulting in the generation of P450s with high amino acid homology and different catalytic specificity. On the other hand, CYP4F4 genes in rat (CYP4F1, 4F4, 4F5 and 4F6) and mouse (Cyp4f13, 4F14, 4F15, 4F16 and 4F18) form a close cluster on chromosome 7q11 and 17b1, respectively (Nelson et al., 2004). Kikuta et al. (1998a) isolated the gene encoding CYP4F3 and determined its genomic organization and chromosomal localization. The CYP4F3 gene contained 13 exons and spanned approximately 22.2 kb. The cDNA of CYP4F3 contained 5050 nucleotides excluding the poly (A) tail. Fluorescence in-situ hybridization demonstrated that the CYP4F3 gene is located at 19p13.2 (Kikuta et al., 1998a). Subsequently, the gene encoding for CYP4F2 was cloned and its genomic organization and the promoter activity was determined (Kikuta et al., 1999b). The structure of CYP4F genes is quite similar across the species (Kikuta et al., 2002). They all contain 13 exons with their open reading frames being encoded from exon 2 to exon 13. In the case of CYP4F2 and 4F3, exon 1 includes 49 bp of a 5′-untranslated region (UTR). The 5′ flanking sequence downstream from −165 of the CYP4F2 gene has 75% similarity to the corresponding...
region of the CYP4F3 gene. Deletion of the 5′ flanking regions containing putative regulating elements recognized by HNF-3β, CDP CR, and p300 caused alterations in the transcriptional activity of CYP4F2 gene (Kikuta et al., 1999b).

4.1. Basal expression and inducibility

A list of known CYP4F subfamily inducers and repressors is shown in Table 1. Zhang et al. (2000) sequenced a 6.7-kb genomic fragment of CYP4F2 gene that included the first 5 exons and 500 bp of the 5′-flanking region. Besides the TATA box at −39 bp and basal transcription factor binding sites, the promoter region and 412-bp intron 1 contain several putative binding sites for nuclear factors that may mediate the inflammatory response and lipid homeostasis (Zhang et al., 2000). However, 5′-deletion analysis has revealed that the TATA sequence identified in the proximal promoter of the CYP4F2 gene is dispensable for its transcription (Kikuta et al., 1999b). Interestingly, no TATA box or TATA-like sequences are present in the 2 kb 5′-flanking regions of the rat CYP4F genes (unpublished results). Instead, CCAAT boxes flanked by Sp1 sites are noticed at positions close to the transcription initiation sites.

CYP genes with TATA-less promoters are not uncommon (Zhao et al., 1995; Cui & Strobel, 2002; Anakk et al., 2003) and it is known that Sp1 sites are crucial in transcription initiation for many TATA-less promoters (Crawford et al., 1999). DNase I footprinting has revealed 3 protected sequences, with the region containing 2 CCAAT boxes at −71 and −111 bp being important in CYP4F2 gene expression (Zhang et al., 2000). Likewise, Cui and Strobel (2002) isolated the 134 bp promoter necessary for the basal expression of CYP4F5, and identified the CCAAT box with Sp1 sites as the most striking feature in this region for its constitutive expression. More subtle deletions and mutations will clarify further whether Sp1 binding sites present in CYP4F genes are functional and which one(s) is/are necessary in transcription.

Zhang et al. (2000) found 2 direct repeat (DR)-1 elements in the 5′ promoter, a DR-2 element in intron 1, and retinoid X receptor (RXR)/retinoid A receptor (RAR) binding sites in both intron 1 and the 5′ promoter of CYP4F2 gene. Transient transfection experiments identified 2 sites in the 5′ promoter and intron 1 that cooperate in gene transcription while exon 1 and a GC-rich region flanking exon 1 inhibit transcription (Zhang et al., 2000). All-trans retinoic acid (ATRA) and 9-cis-retinoic acid (9-cisRA) stimulated promoter activity 3- and 6-folds, respectively, while cotransfection with RXRα or RAR/ RXRα further enhanced activity. Both saturated fatty acids and 12-hydroxydocosanoic acid also stimulated CYP4F2 gene promoter activity (Zhang et al., 2000).

Furthermore, HepG2 cells transfected with reporter constructs of CYP4F2 promoter and treated with either ATRA or 9-cisRA showed a significant increase in reporter activity (Zhang & Hardwick, 2000). The CYP4F2 protein content in HepG2 cells increased with 9-cisRA, but not with ATRA (Zhang & Hardwick, 2000). Cotransfection with RXRα enhanced reporter activity by 2.5-fold, while RXRα/RARα increased activity by 1.5-fold. In contrast, cotransfection with RARα decreased reporter activity by ATRA up to 30%. From these results, the authors concluded that RA can regulate CYP4F2 gene activity through retinoic acid receptors such that RXRα induces while RARα represses CYP4F2 gene expression (Zhang & Hardwick, 2000). The additional 3 retinoic acid response elements identified around the transcription start site of CYP4F2 gene may also affect this induction process.

Zhang et al. (2000) also reported that peroxisome proliferating compounds like Wy14,643 and clofibrate, decrease the CYP4F2 gene activity in HepG2 cells. Unlike members of the CYP4A subfamily, which are induced by hypolipidemic drugs and peroxisomal proliferators (Johnson et al., 1996), the CYP4F are known to be repressed after these treatments (Chen & Hardwick, 1993; Kawashima et al., 1997; Kikuta et al., 2000a; Kalsotra et al., 2002). The activation of CYP4A genes by these agents occurs via activation of the nuclear receptor PPARα (Issemann & Green, 1990) but the mechanism of CYP4F gene suppression by hypolipidemic drugs remains to be determined. The genomic characterization of 4 kb 5′-flanking region, including the 134 bp basal promoter, failed to support PPARα mediated CYP4F5 gene regulation (Cui & Strobel, 2002). In a parallel study using PPARα−/− mice, reduction in hepatic Cyp4f16 expression upon clofibrate administration was found to be PPARα independent (Cui et al., 2001).

Most of the physiological functions of PPAR can be explained by their activity as transcription factors that modulate the expression of specific target genes (Barbier et al., 2004; Evans et al., 2004). PPAR can also repress gene transcription in a DNA-binding-independent manner by interfering with nuclear factor-κB (NF-κB), signal transducer and activator of transcription (STAT), activator protein-1 and CCAAT/enhancer binding proteins (C/EBP) signaling pathways via protein–protein interactions and cofactor competition (Jiang et al., 1998; Chinetti et al., 2000; Gervois et al., 2001; Barbier et al., 2004). Such transrepression mechanisms are likely to participate in the anti-inflammatory actions of PPAR (Barbier et al., 2004). Interestingly, in-silico DNA sequence analysis predicted the presence of putative sites for activator protein-1, NF-κB and C/EBPβ in the CYP4F promoters (unpublished results). The crosstalk between these cofactors and PPAR seems plausible for initiating a general anti-inflammatory response that incorporates the CYP4F subfamily.

4.2. Alternative splicing of CYP4F3 gene

Alternative splicing is being increasingly recognized as a shared theme in CYP gene regulation to generate tissue-specific novel P450s (Nelson et al., 2004). Splicing variations in CYP can either maintain the kinetic properties of the enzyme or generate novel catalytic functions depending upon the positions and sites of splicing. For instance, CYP19 (aromatase) gene is alternatively spliced at the 5′-UTR resulting in no effect on the functional activity of the enzyme (Simpson et al., 1997). Alternatively, a frameshift mutation and alternate splicing in human brain generates a functional form of the pseudogene CYP2D7 that demethylates codeine to morphine (Pai et al.,...
Furthermore, intergenic mRNA molecules resulting from trans-splicing of CYP3A genes can form catalytically active form of chimeric CYP3A protein (Finta & Zaphiropoulos, 2002). These alterations provide additional level of diversity for a group of enzymes that already exhibit remarkable variation in substrate and product specificities. Interestingly there is evidence that CYP4F subfamily undergoes alternative splicing both at their coding regions as well as the 5′-UTR (Christmas et al., 1999; Kikuta et al., 2000a).

The discovery of a splicing event occurring in a CYP4F was made with 4F3 gene. Utilizing elegant biochemical and molecular biology approaches the authors reported that the tissue-specific expression of functionally distinct CYP4F3 isoform is regulated by alternative promoter usage and mutually exclusive exon splicing (Christmas et al., 1999) (Fig. 4). They examined the alternative splicing of exon 3 of the CYP4F3 gene by RT-PCR and demonstrated that a new isoform is generated by selection of alternative promoter usage and mutually exclusive exon splicing (Christmas et al., 1999) (Fig. 4). Selection of exon 4 generates the neutrophil isoform (CYP4F3A), which has a low Km for LTB4 of <1 μM. Selection of exon 3 instead of exon 4 generates an alternative isoform (CYP4F3B), which has a 44-fold lower efficiency of inactivating LTB4 (Christmas et al., 1999; Christmas et al., 2001). Both exons are 145 bp and code for amino acids 67–114 with only 27% amino acid identity.

Christmas et al. (2001) have further illustrated that despite being a predominant CYP4F isoform in human liver and ileum, CYP4F3B is also significantly expressed in kidney suggesting it to be one of the key enzymes to produce 20-HETE. Considering CYP4F2 as another major arachidonate ω-hydroxylase (Lasker et al., 2000), it can be assumed that CYP4F3B and CYP4F2 work together as 20-HETE synthetase, especially in the renal tissue. Interestingly, the primary structure of CYP4F3B shows 94% amino acid homology to that of CYP4F2. Thus, CYP4F3B seems to be quite similar to CYP4F2 in its tissue localization and functional properties. However, in contrast to CYP4F3, the CYP4F2 gene shows no alternative splicing. Reactions and direct sequencing of PCR products have failed to detect a splice form of CYP4F2 containing exon 4 in any of the human tissues (Christmas et al., 2001). While prostate, ileum, and trachea express both splice variants of CYP4F3 gene, these tissues express only a single form of CYP4F2 expressing the exon 3.

CYP4F11 cDNA from brain on the other hand has a 5′-UTR different than the 5′-UTR of the liver form (Cui et al., 2000). The brain 5′-UTR is quite short and is located 300 bp upstream from the liver CYP4F11 exon 1. Kikuta et al. (2000a) have similarly found that Cyp4f14 gene in mouse produces 3 different transcripts with unique 5′-UTR. CYP4F1, an orthologue of Cyp4f14 in the rat, however generates only 2 transcripts that are different in their 5′-UTR (unpublished results). CYP4F1 transcription starts from 2 distinct sites resulting in a 23- or 17-bp 5′-UTR designated as CYP4F1A and CYP4F1B. CYP4F1B has 12 exons in contrast to the 13 exons present in other rat CYP4F. Although CYP4F1A and CYP4F1B code for 1977 and 1972 bp, respectively, both splice variants have the same open reading frame and protein sequence of 524 amino acids (unpublished results). Absence of an intron sequence with high homology to its exon sequences rules out mutually exclusive exon splicing of CYP4F1. The observation that 2 transcription start sites are located in the CYP4F1 gene suggests the possibility of alternative usage of 2 promoters by different tissues or different inducers. However, we have not yet obtained any evidence for this supposition.

4.3. Regulation of CYP4F3 gene in myeloid cells

Christmas et al. (2003) have investigated the mechanisms that regulate cell-specific expression of the 2 alternatively spliced forms of CYP4F3. CYP4F3A mRNA can be detected in cells expressing markers of myeloid differentiation, such as CD11b, and myeloperoxidase during development in the bone marrow suggesting that differentiation to neutrophils may be related to CYP4F3A expression (Christmas et al., 2003). However, known myeloid transcription factors do not display a transcriptional activity for CYP4F3A, and the promoter is activated by Zeb-2 (Christmas et al., 2003), a factor primarily characterized as a transcriptional repressor in the various cells including lymphocytes (Postigo & Dean, 2000; Postigo et al., 2003).

![Diagram](image_url)
In contrast, CYP4F3B expression was shown to be restricted to a small population of CD3+ T lymphocytes. The authors identified distinct transcriptional features in myeloid, lymphoid, and hepatic cells that indicate the presence of multiple promoters in the CYP4F3 gene (Christmas et al., 2003). The hepatic promoter was shown to be dependent on a cluster of hepatocyte nuclear factor sites 123–155 bp upstream of the initiator ATG codon. But it remained unclear whether CYP4F3A-related LTB4 ω-hydroxylase activity is actually induced according to differentiation into neutrophils until Mizukami et al. (2004) examined the time course of CYP4F induction in ATRA-differentiated neutrophils from HL-60 cells. ATRA specifically induced the expression of CYP4F3A in parallel with the induction of the superoxide-generating activity (Mizukami et al., 2004). These observations suggested that the induction of the LTB4 ω-hydroxylating activity was closely related to the ATRA-induced differentiation of HL-60 cells into PMNL.

In a parallel study, Kikuta et al. (2004) demonstrated the expression of CYP4F isoforms in human leukocytes and HL60 cells. Enzymatic activity assay, immunocytochemical staining, and RT-PCR analysis of human leukocytes showed that PMNL express CYP4F3B and CYP4F12 in addition to CYP4F3A. Transcription start site of CYP4F3B mRNA in PMNL expresses CYP4F3B and CYP4F12 in addition to CYP4F3A. Enzymatic activity assay, immunocytochemical staining, expression of CYP4F isoforms in human leukocytes and HL60 cells, but is inoperative in PMNL. Although this promoter is effective in untreated HL60 cells, CYP4F3 expression was never detected in the cells. Furthermore, a fragment of the CYP4F3 gene (−190/+49) showed transcriptional activity in HepG2 cells, in which no CYP4F3 was detected (Kikuta et al., 1999b). These facts suggest that the transcription of CYP4F3 gene is strongly inhibited by a repressor in untreated HL60 cells and hepatocytes, while CYP4F3B is expressed in human liver by using different promoter (Christmas et al., 2003).

4.4. CYP4F12 polymorphism

CYP4F12 cDNA cloning and tissue expression analysis identified 3 major transcripts in liver, kidney, small intestine and colon (Bylund et al., 2001). The presence of 5 additional polyadenylation sites in the 3′-UTR was thought to be responsible for generation of its multiple transcripts. However, presence of multiple transcripts is not unique to CYP4F12 as they are observed for 4F8 and 4F11 as well (Bylund et al., 1999; Cui et al., 2000). In the past year several CYP4F12 genetic polymorphisms were identified from an effort to seek out differences in the 2 clones of CYP4F12 separately described by Bylund et al. (2001) and Hashizume et al. (2001a). There were substitutions in 4 nucleotides (G226 to A, G268 to A, C562 to T and G1564 to A), leading to modification in amino acids namely Asp76 to Asn, Val90 to Ile, Arg188 to Cys in exon 3 and Gly522 to Ser in exon 13, respectively, besides containing the missing N-terminal portion (199 bp).

Polymorphism is defined in simple terms as common variation in the sequence of DNA among individuals and is an important factor responsible for inter-individual variability of CYP genes. It is a crucial aspect since this variability may reflect on the metabolism of endogenous as well as exogenous substrates carried out by the CYP4F enzymes. Lo-Guidice’s group identified several polymorphisms in CYP4F12 gene in 53 unrelated French Caucasians (Cauffiez et al., 2004a). Using Polymerase Chain Reaction-Single Strand Conformational Polymorphism technique, the polymorphisms were determined from their DNA samples. The analysis of the CYP4F12 promoter yielded a total of 12 mutations (Cauffiez et al., 2004a).

The first mutation comprised a large deletion located in intron 1 (CYP4F12*1v), followed by 2 isolated substitutions −402G>A (CYP4F12*3v) and −188T>C (CYP4F12*4v) and finally 9 combined mutations, −474T>C, −297A>C, −224A>G, −173G>A, −145C>G, −140T>C, −126T>C, −56T>C, and −21T>G (CYP4F12*2v) (Cauffiez et al., 2004a). Considering the nature and location of the polymorphisms for CYP4F12*1v and *2v, the functional relevance of those 2 allelic variants was further examined by transfecting different cell lines with constructs of the related region of the CYP4F12/luciferase reporter gene. CYP4F12*1v and *2v lead to a significant decrease of CYP4F12 gene expression in HepG2 cell line (Cauffiez et al., 2004a). Interestingly the first 192 bp of the 5′-UTR in CYP4F12 gene carries putative binding sites for RAR/RXR, NF-κB, Sp1 and GATA and this fragment of the gene is deleted in CYP4F12*1v. Also, the mutations present in CYP4F12*2v cause loss of binding sites for hepatocyte nuclear factor, C/EBPα and GATA-1 (Cauffiez et al., 2004a). The loss of these sites might explain the 50% decrease in promoter activity of CYP4F12*1v and *2v in HepG2 cells suggesting the importance of these elements in maintaining the transcription of CYP4F12 wild type gene.

In addition to the promoter, the CYP4F12 coding region was also found to be polymorphic (Cauffiez et al., 2004b). A set of 10 polymorphisms identified in the CYP4F12 coding region comprised 7 mis-sense mutations, 31C>T (Leu11Phe), 38C>T (Pro13Leu), 47C>T (Met16Thr), 4759G>A (Aqp76Asn),
4801G>A (Val90Leu), 8896C>T (Arg188Cys) and 23545G>A (Gly522Ser). Amongst these 38C>T in exon 2, 8896C>T and 23545G>A in exon 6 were the most frequent mutations with a frequency of 25%, 47% and 41% respectively. Two out of these 7 variants (Val90Ile and Arg188Cys) displayed changes in CYP4F12 catalytic activity with significant alterations in their Michaelis-Menton (K_m, V_max) parameters (Cauffiez et al., 2004a). These 2 studies have established the functional impact of polymorphisms in both the regulatory as well as coding region of CYP4F12 gene. It will be interesting to see if other CYP4F contain similar polymorphic alleles as it may cause serious implications in endogenous and xenobiotic metabolism supported by CYP4F subfamily.

5. In-vivo and in-vitro regulation of rodent cytochrome P450 4F during inflammation

Although inflammation is an integral part of the host defense mechanism against a variety of pathogens as well as tissue injury, it often produces a myriad of unwanted complications. Immediately after a tissue injury or bacterial infection, PMNL are activated and recruited at the site of inflammation by soluble mediators such as LT_B4 causing cell adherence, transcapillary migration and chemotaxis (Springer, 1994; West et al., 2005). The liver form of CYP4F3, which is generated by alternative splicing (Christmas et al., 1999), and another human CYP4F, CYP4F2 (Kikuta et al., 1994), are thought to control excessive inflammation by removal of LT_B4 from human liver, a major organ for its inactivation (Keppler, 1992; Christmas et al., 2001). One way to provide support for this hypothesis is to test whether the hepatic expression of CYP4F changes during an inflammatory response, since these changes are required to adjust the levels of inflammatory mediators.

5.1. Hepatic and renal regulation of cytochrome P450 4F upon endotoxin treatment

Administration of bacterial endotoxin lipopolysaccharide (LPS) is extensively used as a model of sepsis to study inflammatory responses during infection (Hornef et al., 2002; Raetz & Whitfield, 2002), and is known to increase LT_B4 production (Doerfler et al., 1989; Rankin et al., 1990; Surette et al., 1998). Similar to LPS, barium sulfate (BaSO4) can cause a systematic inflammatory response, but remains different in some aspects. First, LPS injection can directly activate Kupffer cells and cause a high cytokine accumulation in the liver as compared with rats which receive BaSO4 injected intraperitoneally (Sewer et al., 1997). In addition, injection of BaSO4, a particulate irritant, is thought to initiate a sterile peritonitis following phagocytosis by macrophages (Ufkes et al., 1988) or by Kupffer cells after the particulate enters the blood circulation, and is transported to liver (Peterson & Renton, 1986).

Recent studies have shown that CYP4F are regulated in a tissue- and isoform-specific manner in LPS- and BaSO4-treated inflammatory models indicating that distinct mechanisms in CYP4F regulation operate under different inflammatory prompts (Cui et al., 2003; Kalsotra et al., 2003a). In liver, LPS and BaSO4 do not affect the expression of CYP4F1 and 4F6, 2 major CYP4F isoforms. LPS administration produces opposite effects on CYP4F4 and 4F5 expression causing a down-regulation of 4F4 and up-regulation of 4F5 (Kalsotra et al., 2003a). On the other hand, BaSO4 treatment increases CYP4F4 expression. In kidney, none of the rat CYP4F isoforms are affected by LPS treatment, however, BaSO4 induces the expression of CYP4F1 and 4F6 (Kalsotra et al., 2003a). Since no significant change in net LT_B4 metabolism after LPS treatment is evident by liver microsomes (Kalsotra et al., 2003a), the opposite regulation of CYP4F4 and 4F5 are suggested to cancel out one another, leaving no net change in activity. In contrast, BaSO4 treatment causes a modest increase in LT_B4 metabolism which correlates with the increased CYP4F4 mRNA expression observed upon BaSO4 challenge (Kalsotra et al., 2003a). The differential regulation observed in kidney and liver indicates that CYP4F might have a separate function other than LT_B4 inactivation in the kidney. This is consistent with the notion that CYP4F enzymes are among the major catalysts for 20-HETE generation in human as well as rodent kidneys (Lasker et al., 2000; Christmas et al., 2001; Kroetz & Xu, 2004; Xu et al., 2004).

To elucidate the regulatory mechanism of the CYP4F expression during inflammation, renal and hepatic Cyp4f15 and Cyp4f16 expression were analyzed using wild-type (SV/129) mice and PPARα null mice with or without challenge by LPS or clofibrate (Cui et al., 2001). Renal expression of Cyp4f15 is induced by LPS and clofibrate in (+/+) mice, and these effects are absent in the (−/−) mice. Renal expression of Cyp4f16 is not affected by LPS or clofibrate in (++) or (−/) mice. In contrast, hepatic expression of Cyp4f15 and Cyp4f16 is significantly reduced by LPS-treatment in (++) mice. A lesser reduction is also seen in the (−/) mice, suggesting that PPARα is partially responsible for this down-regulation. Likewise, a recent report by Richardson et al. has shown that mice infected with Citrobacter rodentium, a rodent equivalent of human enteropathogenic Escherichia coli infection, causes a significant reduction in hepatic Cyp4f14 and 4f15 mRNA levels while inducing 4f16 and 4f18 mRNA levels (Richardson et al., 2006). Cyp4f15 response was shown to be toll-like receptor 4 dependent (Richardson et al., 2006), a cell surface receptor responsible for generating host inflammatory response to various invading pathogens (Poltorak et al., 1998).

5.2. Cytokine mediated effects on cytochrome P450 4F expression in isolated hepatocytes

In order to dissect the alterations in hepatic CYP4F expression as direct or indirect effects of LPS, CYP4F regulation has been studied in cultured rat hepatocytes subjected to varying concentrations of LPS. All 4 isoforms display a decrease upon LPS challenge (Kalsotra et al., 2003a). The effects of LPS on CYP mRNAs are known to be mimicked by administration of interferon inducers and cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α (Morgan, 1991; Morgan et al., 1994; Chen et al., 1995; Renton, 2001), hence their contribution in CYP4F regulation was tested.
In the early stages of an inflammatory response, these cytokines are produced by monocytes/macrophages and endothelial cells and are released into the systemic circulation, initiating the so-called acute-phase response (Baumann & Gauldie, 1994; Fan & Malik, 2003; Karaghiosoff et al., 2003). Hepatocytes are influenced by these inflammatory cytokines and dramatically alter the synthesis of a number of CYP (Morgan, 1997, 2001).

Interestingly, the results obtained from hepatocyte treatment with IL-1β were quite similar to the in vivo effects of LPS on CYP4F expression (Kalsotra et al., 2003a). This implies that perhaps intraperitoneally injected LPS causes increased production of cytokines such as IL-1 which directly affect CYP4F expression in liver. IL-1 has been shown to mediate its pro-inflammatory effects through NF-κB, a dimeric transcription factor formed by the hetero or homo-dimerization of proteins in the Rel family, including p50 and p65 (Baldwin, 1996). CYP4F5 gene contains NF-κB binding sites in the promoter as well as 5′ flanking region (Cui & Strobel, 2002); however, the functionality of these sites remains to be tested.

We have recently determined that pro-inflammatory cytokines IL-6 and TNF-α produce a general inductive response whereas IL-10, an anti-inflammatory cytokine, suppresses CYP4F expression (unpublished results). The molecular mechanism behind IL-6 related induction of CYP4F4 and 4F5 is partially signal transducer and activator of transcription 3 dependent. Therefore the up regulation of CYP4F isoforms by IL-6 are, to a certain extent, controlled by signal transducer and activator of transcription 3 dimerization and subsequent nuclear translocation (Kaptein et al., 1996; Taga & Kishimoto, 1997). In addition to IL-6, TNF-α is also able to induce CYP4F; however, the timing of induction is more rapid with TNF-α.

IL-10 on the other hand produces significant decrease in CYP4F expression (unpublished results). These findings are especially interesting since IL-10 is a pleiotropic cytokine that controls many inflammatory processes (Bogdan & Nathan, 1993; Kuhn et al., 1993; Moore et al., 2001). The anti-inflammatory effects of IL-10 are achieved through the suppressed production of inflammatory proteins such as IL-1, IL-6, IL-8, IL-12, TNF, granulocyte-macrophage colony stimulating factor and granulocyte colony stimulating factor (Bogdan & Nathan, 1993; Moore et al., 1993; Fadok et al., 1998). Thus, the inhibitory effect on CYP4F expression caused by IL-10 could be due to the suppression of the constitutive pathways, which are directed by these factors and are responsible for basal expression of CYP4F. On the other hand, it is reasonable that IL-10 exerts a direct transcriptional control on CYP4F gene regulation. Nevertheless, the opposite regulation of CYP4F by pro- and anti-inflammatory cytokines implies a critical function for these enzymes.

5.3. Cytochrome P450 4F expression in brain in response to traumatic brain injury

An alternate avenue of triggering the inflammatory cascade is through a traumatic brain injury (TBI) model. The TBI model in rat closely simulates closed head injury in humans who have been in traffic accidents or blunt trauma (Dixon et al., 1991). Apart from mechanical disruption of brain tissue and vasculature, the extracellular and intracellular changes that follow have been termed “secondary injury”. These secondary events occur within hours or days after primary injury, and can lead to further damage of the nervous system (Povlishock & Christian, 1995; Feuerstein et al., 1997). Secondary injury initiates excitotoxicity, which arises due to the excessive release of neurotransmitters, influx of ions and production of inflammatory mediators (Faden et al., 1989; Xu et al., 1990; McIntosh et al., 1999). Analysis of brain tissue following impact in comparison to sham-treated controls offers a model of the injury-prompted inflammatory cascade in the absence of a systemically introduced prompt.

Cui et al. (2003) have shown that brain injury brings about a significant change in the hippocampal expression of CYP4F4 and 4F5 mRNA which reaches its greatest effect 24 hr after impact compared with sham-operated but uninjured controls. At time points after 24 hr the expression of both isoforms increases reaching highest levels at 2 weeks post-injury. These changes in mRNA expression are mirrored by changes in protein expression (Cui et al., 2003). The results are consistent with the notion that immediately after injury, concentrations of LT and PG mediators are elevated by decreased CYP4F concentrations (Xu et al., 1990; Cui et al., 2003). As time after injury increases those conditions reverse. Increased CYP4F expression is suggested to reduce concentrations of LT and PG leading to recovery and repair.

5.4. Extracranial induction of cytochrome P450 4F after traumatic brain injury

Unfortunately, trauma to the brain, besides causing damage to the primary site of insult, leads to multiorgan dysfunction syndrome (Ott et al., 1994; Gruber et al., 1999; Campbell et al., 2003). This results in a myriad of pathophysiological complications affecting neurological, psychological, cardiovascular, pulmonary, vascular and metabolic functions, which in turn impede the recovery process (Clifton et al., 1983; Fisher et al., 1999; McIntosh et al., 1999; Kalsotra et al., 2003b). To test whether TBI produces any effects on hepatic and renal CYP4F enzymes, their expression and activity analysis was carried out (Kalsotra et al., 2003b). The liver CYP4F levels do not alter appreciably upon brain injury but a significant up-regulation is evident in the kidney (Kalsotra et al., 2003b; Strobel et al., 2003). From these observations, one could speculate that changes in CYP4F driven 20-HETE production in kidney might contribute to the renal effects associated with brain trauma.

The association between brain injury and subsequent pulmonary dysfunction has been recognized clinically. Although the risk of developing lung injury subsequent to head trauma does not necessarily correlate with episodes of severe hypoxia (Holland et al., 2003), several pieces of evidence suggest it may occur as a result of a systemic inflammatory response (De Simoni et al., 1990; Morganti-Kossman et al., 1997; Woiczechowsky et al., 1998; Gabay & Kushner, 1999; Woiczechowsky et al., 2002; Stubble et al., 2004). Fisher et al. (1999) have reported enhanced pulmonary inflammation in
organ donors following fatal brain injury. This severe inflammatory response stems from increased cytokine production and infiltration of activated neutrophils into the lung, which provides the potential for massive localized tissue injury.

In accordance with the study of Fisher et al. (1999), we have observed that brain injury causes large migration of inflammatory cells (especially macrophages and neutrophils) in the major airways and alveolar spaces at 24 hr post-injury, which is associated with enhanced pulmonary LTB₄ production within the lung (unpublished results). However, by 2 weeks after injury, a temporal switch has occurred and the resolution of inflammation is underway. We find that 5-LOX and CYP₄F, the respective enzymes responsible for LTB₄ synthesis and breakdown, play crucial roles in setting the cellular concentration of LTB₄ (unpublished results). Activation of LTB₄ breakdown via induction of CYP₄F, predominantly in the lung tissue, serves as an endogenous signal to ameliorate further secondary damage (Strobel et al., 2003). In addition, we show that CYP₄F are localized primarily in the airways and pulmonary endothelium (unpublished results). Adherence of circulating neutrophils to the microvascular endothelium is the initial step in diapedesis, the process by which leukocytes migrate through blood vessels to accumulate at sites of infection or injury (Springer, 1994). LTB₄ clearance by CYP₄F in the endothelium may be an ideal site to resolve pulmonary inflammation initiated after brain injury.

Taken together these studies provide evidence that in the pro-inflammatory phase, increased local production of LTB₄ leads to mobilization of inflammatory cells to the extracranial tissues. In the resolution phase, CYP₄F expression and associated activity increases with time causing a decline in LTB₄ concentration to the normal cellular levels. This marks a significant reduction in neutrophil recruitment which leads to subsequent recovery and repair. Thus, CYP₄F present a novel checkpoint in inflammation control that may be targeted for early treatment of inflammation following brain injury. The model presented in Fig. 5 illustrates 2 specific time points, 24 hr and 2 weeks after TBI, where there is a major switching from pro-inflammatory to anti-inflammatory processes.

6. Cytochrome P₄₅₀ 4F participation and impact on drug metabolism

CYP have been characteristically termed as a family of drug metabolizing enzymes. This aspect of CYP has been typically attributed to CYP₃A which metabolize more than 50% of drugs, followed by CYP₂D, CYP₁A and CYP₂B subfamilies (Gonzalez, 1992; Guengerich, 1999; Nebert & Russell, 2002). Human CYP₄F isoforms are known to metabolize endogenous eicosanoids; however recently some 4F members have also gained recognition in metabolizing clinically active drugs.

6.1. CYP₄F₁₁

Catalytic characterization of CYP₄F₁₁ revealed its capability to catalyze N/O demethylation of many known P₄₅₀ drug substrates (Kalsotra et al., 2004). Erythromycin, a probe substrate for CYP₃A subfamily (Watkins et al., 1989; Thummel & Wilkinson, 1998) was efficiently turned over by CYP₄F₁₁. The kinetic analysis showed that CYP₄F₁₁ had an apparent $V_{\text{max}}$ of 830 pmol/min/nmol P₄₅₀ and $K_{\text{m}}$ of 125 μM for erythromycin which are in the same range of values as CYP₃A4. The same study demonstrated that CYP₄F₁₁ in comparison to CYP₄F₃A lacks the ability to metabolize eicosanoids (Kalsotra et al., 2004). While CYP₄F₃A metabolized LT₄, lipoxins and HETE much more efficiently than 4F₁₁, CYP₄F₁₁ was a better catalyst for many drug substrates such as benzphetamine, ethylmorphine, chlorpromazine, and imipramine (Kalsotra et al., 2004). In addition, CYP₄F₁₁ converted chlorpromazine into nor-chlorpromazine and chlorpromazine-sulfoxide.
To define some of the possible structural reasoning for such a difference in substrate specificity between the 2 closely related CYP4F isozymes, 3 dimensional homology modeling of the 4F11 and 4F3A proteins was carried out (Kalsotra et al., 2004). The 2 proteins have 82.3% amino acid sequence identity (Cui et al., 2000) and have fairly similar secondary structures but there are some key differences in their substrate access channels. CYP4F3A displays a much more restricted substrate access channel than 4F11 which may explain the lower turnover rates observed for 4F3A with certain drugs (Kalsotra et al., 2004). Compared to CYP4F11, 4F3A is predominantly hydrophobic in the core, which may limit the access of hydrophilic drug substrates while facilitating the interaction of hydrophobic eicosanoids with this region.

Structural homology modeling of the 2 proteins also revealed some interesting differences in the substrate recognition site 2, an important structural determinant influencing P450 catalysis (Gotoh, 1992; Peterson & Graham-Lorence, 1995). The model of CYP4F11 presents a more open access channel that may explain the ability to metabolize large molecules like erythromycin. Also, some wide variations in residue size, charge, and hydrophobicity in the FG loop region may contribute to the differences in substrate specificity and activity seen between CYP4F3A and CYP4F11.

6.2. CYP4F12

Ebastine, a novel antihistamine pro-drug is broken down via first pass metabolism to 2 major metabolites in human microsomes. The inactive metabolite desalkyl ebastine is obtained via dealkylation and the active drug entity carebastine is obtained via hydroxylation. CYP3A was identified to catalyze the dealkylation pathway whereas the enzyme responsible for hydroxylation remained unknown (Hashizume et al., 1998). Using specific inhibitors to the different CYP subfamilies, Hashizume et al. (2001b) identified CYP4F subfamily as one of the key mediators of this above mentioned process. Ebastine hydroxylase was primarily carried out by CYP4F family member as shown by the fact that incubation with CYP4F antibody reduced the formation of carebastine by recombinant CYP4F11 up to 85% (Hashizume et al., 2002).

6.3. Rodent cytochrome P450 4F

Rat CYP4F are also active in metabolizing both endogenous autacoids and drugs. For instance, recombinant CYP4F6, which has low catalytic activity towards typical 4F substrates from the eicosanoid group (Xu et al., 2004), converts imipramine to its 10-hydroxy product (Kawashima et al., 1996). In addition, Boehme and Strobel (2001) have shown that both CYP4F4 and 4F5 metabolize chlorpromazine to the N-demethylated and the S-oxide products. Thus CYP4F may play a dual enzymatic role, one in catalyzing eicosanoid breakdown and other in carrying out drug metabolism.

7. Concluding remarks

Advances in molecular biology and genomics facilitated the biochemical characterization of individual CYP4F enzymes, which in turn revealed many surprises about an enzyme system once believed to metabolize only LTβ. Today, the biological functions of CYP4F include metabolism of several other pro-inflammatory eicosanoids.

There is a worldwide consensus in the scientific community that acute inflammation followed by a timely resolution plays a fundamental part in the body’s response to trauma, tissue injury as well as to microbial infection. Lipid-derived mediators, particularly PG and LT, are pivotal in orchestrating inflammation and have been targeted by the pharmaceutical industry for anti-inflammatory therapies. These drugs are used extensively and appear to be effective in many clinical settings; however, considerable controversy remains about their general safety and unwanted side effects in the gastrointestinal and cardiovascular systems. As covered in this review, the timely clearance of LTβ by CYP4F appears to be of physiological relevance for the resolution of inflammation. However, the significance of LTβ catabolic pathway in controlling inflammation will be better established by studying LTβ metabolism in a CYP4F knockout background that could bring forward a previously unappreciated alternative to the existing anti-inflammatory regimen and may be with fewer side effects than the inhibitor approach currently used.

Lastly, the fact that both rodent and human CYP4F are able to metabolize clinical drugs implies that the 4F subfamily may carry out 2 fold function in the management of inflammatory diseases, one by inactivating eicosanoids and other by serving as alternate “overload” metabolism enzymes for cases when excess drug toxicants are encountered, or in some cases where structures of the xenobiotics closely resemble the eicosanoids.

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