Catalytic Activity and Isoform-Specific Inhibition of Rat Cytochrome P450 4F Enzymes

Fengyun Xu, John R. Falck, Paul R. Ortiz de Montellano, and Deanna L. Kroetz

Departments of Biopharmaceutical Sciences (F.X., D.L.K.) and Pharmaceutical Chemistry (P.R.O.d.M., D.L.K.), University of California San Francisco, San Francisco, California; and Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas (J.R.F.)

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ABSTRACT

Arachidonic acid is \(\omega\)-hydroxylated to 20-hydroxyeicosatetraenoic acid (20-HETE), which has effects on vasoactivity and renal tubular transport and has been implicated in the regulation of blood pressure. Cytochrome P450 (P450) 4A isoforms are generally considered the major arachidonic acid \(\omega\)-hydroxylases; however, little is known about the role of rat CYP4F isoforms in 20-HETE formation. The rat CYP4F isoforms, CYP4F1, CYP4F4, CYP4F5, and CYP4F6, were heterologously expressed in \textit{Escherichia coli}, and their substrate specificity in fatty acid metabolism was characterized. Substrate-binding assays indicated that leukotriene \(\beta_4\) (LT\(\beta_4\)) and arachidonic acid bound CYP4F1 and CYP4F4 in a type-I manner with a \(K_m\) of 25 to 59 \(\mu\)M, and lauric acid bound CYP4F4 poorly. Reconstituted CYP4F1 and CYP4F4 catalyzed the \(\omega\)-hydroxylation of LT\(\beta_4\) with a \(K_m\) of 24 and 31 \(\mu\)M, respectively, and CYP4F5 had minor activity in LT\(\beta_4\) metabolism. Importantly, CYP4F1 and CYP4F4 catalyzed the \(\omega\)-hydroxylation of arachidonic acid with an apparent \(k_{cat}\) of 9 and 11 min\(^{-1}\), respectively. Lauric acid was a poor substrate for all of the CYP4F isoforms, and CYP4F6 had no detectable fatty acid \(\omega\)-hydroxylase activity. The P450 \(\omega\)-hydroxylase inhibitors 17-octadecynoic acid, 10-undecynyl sulfate, and \(N\)-methylsulfonyl-12,12-dibromododec-11-enamide showed isoform-specific inhibition of CYP4F1 and CYP4F4-catalyzed \(\omega\)-hydroxylation of arachidonic acid and potency differences between the CYP4A and CYP4F isoforms. These data support a significant role for CYP4F1 and CYP4F4 in the formation of 20-HETE and identify P450 inhibitors that can be used to understand the relative contribution of the CYP4A and CYP4F isoforms to renal 20-HETE formation.

Members of the cytochrome P450 (P450) 4F subfamily have been recently cloned from human, rat, mouse, and ram and are of interest because of their role in the metabolism of endogenous fatty acids, prostaglandins, and leukotrienes (Chen and Hardwick, 1993; Kikuta et al., 1993; Kawashima and Strobel, 1995; Cui et al., 2000, 2001; Bylund and Oliw, 2001; Hashizume et al., 2001). Four isoforms have been identified in the rat to date (Chen and Hardwick, 1993; Kawashima and Strobel, 1995). The first member of this family to be discovered was CYP4F1, a protein that is constitutively expressed at relatively high levels in rat hepatoma (Chen and Hardwick, 1993). Yeast-expressed CYP4F1 catalyzed the \(\omega\)-hydroxylation of leukotriene \(\beta_4\) (LT\(\beta_4\)), lipoxin \(\alpha_1\), prostaglandin \(\alpha_1\), and several hydroxyeicosatetraenoic acids (HETEs) (Kikuta et al., 1993). Rat CYP4F4, CYP4F5, and CYP4F6 were cloned from rat brain (Kawashima and Strobel, 1995). \textit{Escherichia coli}-expressed CYP4F5 had low \(\omega\)-hydroxylase activity toward LT\(\beta_4\), whereas the solubilized membrane fraction of CYP4F4 recombinant protein catalyzed the \(\omega\)-hydroxylation of prostaglandin \(\alpha_1\), prostaglandin \(E_1\), \(6\)-\textit{trans-LTB}_4, and LT\(\beta_4\) (Kawashima and Strobel, 1995). More recently, yeast-expressed CYP4F5 was found to convert LT\(\beta_4\) primarily into \(18\)-hydroxy-LT\(\beta_4\), and a relatively small amount of \(19\)- and \(17\)-hydroxy-LT\(\beta_4\) and yeast-expressed CYP4F6 was found to convert LT\(\beta_4\) into \(19\)- and \(18\)-hydroxy-LT\(\beta_4\) (Bylund et al., 2003). Neither CYP4F4 nor CYP4F1 was able to metabolize arachidonic acid or lauric acid, two typical substrates for CYP4A enzymes (Kawashima et al., 1997; Kikuta et al., 1999). Of the five human CYP4F isoforms identified to date, CYP4F2, CYP4F3, and CYP4F12 have all been shown to catalyze the \(\omega\)-hydroxylation of arachidonic acid (Lasker et al., 2000; Christmas et al., 2001; Hashizume et al., 2001). Moreover, CYP4F2 is the major enzyme that

**ABBREVIATIONS:** P450, cytochrome P450; LT\(\beta_4\), leukotriene \(\beta_4\); HETE, hydroxyeicosatetraenoic acid; MS-PPOH, \(N\)-methylsulfonyl-6-(2-propargyloxyphenyl)hexanoic acid; DDMS, \(N\)-methylsulfonyl-12,12-dibromododec-11-enamide; 17-ODYA, 17-octadecynoic acid; 10-SUYS, sodium 10-undecynyl sulfate; ABT, 1-aminobenzotriazole; HPLC, high-performance liquid chromatography; bp, base pair(s).
catalyzes the ω-hydroxylation of arachidonic acid in human kidney microsomes (Lasker et al., 2000). The ω-hydroxylation of arachidonic acid to form 20-HETE is of increasing interest due to the biological effects of 20-HETE on vascular tone and renal function. 20-HETE inhibits a large conductance, Ca2+-activated K+ channel (maxi K) in renal microvessels leading to potent vasoconstriction (Zou et al., 1996). In addition, 20-HETE is an endogenous inhibitor of Na+,K+-ATPase (Schwartzman et al., 1985), blocks a 70-pS potassium channel in the thick ascending limb of Henle’s loop, and inhibits Na+–K+–2Cl− cotransport (Escalante et al., 1994; Wang and Lu, 1995). 20-HETE has also been implicated in the autoregulation of renal blood flow and tubuloglomerular feedback (Zou et al., 1994a,b). Alterations in renal 20-HETE production may contribute to the development of hypertension in both the spontaneously hypertensive rat and the Dahl salt-sensitive rat (Stec et al., 1996; Kroetz et al., 1997). Inhibition of renal 20-HETE formation with specific fatty acid ω-hydroxylase inhibitors or antisense oligonucleotides reduces blood pressure in the spontaneously hypertensive rat and angiotensin II models of hypertension (Su et al., 2002). Gated.

The biological functions of 20-HETE are largely characterized in the rat, and its renal formation is generally considered to be catalyzed by CYP4A enzymes (Nguyen et al., 1999; Hoch et al., 2000). However, the recent identification of CYP4F isoforms expressed in the rat kidney (Kalsotra et al., 2002; Bylund et al., 2003) and the finding that CYP4F2 is the major arachidonic acid ω-hydroxylase in human kidney (Lasker et al., 2000) led us to hypothesize that the CYP4F enzymes also contribute to renal 20-HETE formation in the rat. In the present study, the four rat CYP4F isoforms were heterologously expressed in E. coli, and their metabolism of fatty acids was characterized. The sensitivity of each of the CYP4F isoforms to known P450 inhibitors was also investigated.

Materials and Methods

Materials. [1-14C]Lauric acid (55 mCi/mmol) and [1-14C]arachidonic acid (56 mCi/mmol) were obtained from Amersham Life Science (Arlington Heights, IL). LTB4 was purchased from Cayman Chemical (Ann Arbor, MI). Human cytochrome P450 reductase was expressed and purified as previously reported (Dierks et al., 1998). Ampicillin, 1-Aminobenzotriazole (ABT) and 17-octadecenoic acid (OA), 1 nm sodium dithionite, all four proteins exhibited a strong absorp-
tion spectra in the oxidized ferric state con-
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CYP4F1 and CYP4F4 were incubated for 30 min at 37°C with 1 mM NADPH, an isocitrate dehydrogenase regenerating buffer as described above, and various concen-
trations of the known P450 inhibitors MS-POH, 17-ODYA, DDMS, 10-SUYS, or ABT. After inactivation, the proteins were diluted 10-fold, and arachidonic acid metabolism was measured as described above. IC50 values were estimated by fitting the data to the equation V = Vc / (1 + (IC50\(^n\))), where Vc is the 20-HETE formation rate in the presence of inhibitor, V is 20-HETE formation rate, I is the inhibitor concentration, and n is the Hill coefficient. The KaleidaGraph fitting program (Abelbeck/Synergy, Reading, PA) was used to fit the data by nonlinear regression.

Results

CYP4F Protein Expression. The CYP4F1, CYP4F4, CYP4F5, and CYP4F6 cDNAs were expressed in E. coli, and the histidine-tagged proteins were purified by Ni\(^{2+}\)-nitrilotriacetic acid agarose affinity column chromatography. Western immunoblotting of the purified proteins with an anti-histidine antibody confirmed that these proteins were histidine-tagged and that the size was in the range of P450 enzymes (data not shown). The proteins, after desalting, exhibited absorption spectra in the oxidized ferric state consistent with their identification as heme-containing P450 enzymes (Fig. 1, A-D). The four proteins exhibited Soret maxima at 418 or 422 nm as expected for low-spin proteins with a distal water ligand to the heme iron atom (Dawson and Sonn, 1987). After reaction with CO and reduction with sodium dithionite, all four proteins exhibited a strong absorption with a maximum at 450 nm, as expected for intact P450
CYP4F1, CYP4F5, and CYP4F6 were present as pure intact, thiolate-ligated P450 proteins. A minor cytochrome P420 shoulder was seen for CYP4F4, indicating this protein was present as a mixture of the active P450 protein and a denatured inactive protein in which the absorption maximum was at 420 nm. The proportion of the protein in the intact, active state in CYP4F4 was about 83%. The CYP4F4 protein has not previously been obtained in purified, active form (Kawashima et al., 1997).

The spectroscopic binding constants for LTB4, lauric acid, and arachidonic acid are summarized in Table 1. All the difference spectra were type I (Estabrook et al., 1972), indicating a net shift of the heme from the low to the high spin state. The spectral perturbation was characterized by a maximum at 390 nm, a trough with an absorbance minimum at 420 nm, and an apparent isosbestic point at 407 nm (Fig. 2). No perturbation was observed when lauric acid was titrated into CYP4F1 or when all three substrates were titrated into CYP4F5 and CYP4F6, suggesting that these fatty acids were bound very poorly or, when bound, did not induce a spin state change of the iron atom. In general, arachidonic acid and LTB4 had similar binding affinity to CYP4F1 and CYP4F4. Lauric acid bound CYP4F4 but not CYP4F1. In contrast, arachidonic acid and lauric acid had higher binding affinity to CYP4A isoforms than CYP4F isoforms (Hoch et al., 2000).

Fatty Acid Metabolism by CYP4F Isoforms. To establish optimal conditions for determination of CYP4F catalytic activity, the effects of varying amounts of NADPH P450 reductase and cytochrome b5 on arachidonic acid hydroxylation were measured. CYP4F1 and CYP4F4 had catalytic activity toward arachidonic acid hydroxylation were measured. CYP4F1 and CYP4F4 had catalytic activity toward arachidonic acid in the absence of cytochrome $b_5$, but an equimolar amount of cytochrome $b_5$ and a 2- to 10-fold excess of NADPH P450 reductase were needed for maximal catalytic activity of both enzymes (data not shown). Based on these preliminary studies, 50 pmol/ml of purified enzyme and a 1:10:1 ratio of CYP4F/cytochrome P450 reductase/cytochrome $b_5$ were chosen to characterize the activity of rat CYP4F isoforms in the metabolism of fatty acids.

CYP4F enzymes have been characterized as LTB4 ω-hy-
droxylases (Kawashima et al., 1997; Kikuta et al., 1999), so this substrate was used as a positive control to test the catalytic activity of the expressed rat CYP4F isoforms. Recombinant CYP4F1, CYP4F4, and CYP4F5 proteins all catalyzed the metabolism of LTB₄, whereas CYP4F6 had no detectable activity (Fig. 3). CYP4F4 had the highest LTB₄ ω-hydroxylase activity with a $K_m$ and $V_{max}$ of 31 $\mu$M and 40 nmol/min/nmol, respectively, followed by CYP4F1 with a $K_m$ and $V_{max}$ of 24 $\mu$M and 10 nmol/min/nmol, respectively (Fig. 4). CYP4F5 had only minor activity toward LTB₄, and the kinetic parameters were not measurable.

Figure 5 shows a representative HPLC chromatogram of arachidonic acid metabolites formed after incubation with the expressed rat CYP4F isoforms. CYP4F1 and CYP4F4 ω-hydroxylated arachidonic acid to form 20-HETE, the major metabolite detected. Both enzymes also formed minor amounts of the ω-1 hydroxylation product, 19-HETE (Fig. 5, A and B). The relative ratios of the ω- and ω-1-hydroxylated metabolites were 21:1 for CYP4F1 and 20:1 for CYP4F4, indicating a strong preference for oxidation at the ω-position of arachidonic acid. In contrast, CYP4F5 and CYP4F6 had no detectable catalytic activity toward arachidonic acid despite modification of the reconstitution and incubation conditions (Fig. 5, C and D).

The kinetics describing arachidonic acid metabolism by CYP4F1 and CYP4F4 were investigated, and the concentration-dependent activity is shown in Fig. 6. Increasing ω-hydroxylase activities were measured with increasing arachidonic acid concentrations up to 40 $\mu$M. Further increases in the concentration of arachidonic acid resulted in a gradual drop in activity. This decrease in activity may be caused by substrate or product inhibition, by micellar aggregation of the fatty acids, or by physical effects of the fatty acids on the reconstituted enzyme. It has previously been suggested that the detergent activity of long-chain fatty acids may disturb the arrangement of the electron transfer partners in the reconstituted system (Hoch et al., 2000).

The catalytic activities of the CYP4F proteins with fatty acids were further characterized by measuring apparent $k_{cat}$ values. CYP4F4 had the highest activity in the metabolism of arachidonate, laurate, and LTB₄ (Table 2). LTB₄ was the best substrate for CYP4F4, whereas arachidonic acid was the best substrate for CYP4F1. Arachidonic acid was a much better substrate for CYP4F1 and CYP4F4 than lauric acid. In contrast, CYP4A1, CYP4A2, CYP4A3, and CYP4A8 all catalyze the ω- and ω-1-hydroxylation of arachidonic acid and lauric acid, with the latter being the preferred substrate (Nguyen et al., 1999; Hoch et al., 2000). CYP4F1 and CYP4F4 had similar arachidonic acid ω-hydroxylase activity as CYP4A1, the most active CYP4A isoform with an apparent $k_{cat}$ of 6 min⁻¹ (Hoch et al., 2000).

Lauric acid, although not physiologically significant, has been used as a prototype substrate for the CYP4A proteins and was investigated here as a substrate for the rat CYP4F isoforms. Only CYP4F4 had minor lauric acid hydroxylase activity (Fig. 7 and Table 2). The relative ratio of the ω- and (ω-1)-hydroxylated metabolites was 5:1.

**Effect of Inhibitors on Recombinant CYP4F-Catalyzed Arachidonic Acid ω-Hydroxylation.** Numerous inhibitors of arachidonic acid ω-hydroxylation and epoxidation have been characterized for their activity against the CYP4A isoforms, but their effects on the CYP4F enzymes have not been reported. The terminal acetylenic compound MS-PPOH selectively inhibited microsomal arachidonic acid epoxidation (Wang et al., 1998), and the addition of MS-PPOH (1–500 $\mu$M) had no effect on CYP4F1- and CYP4F4-mediated ω-hydroxylation of arachidonic acid (Table 3). Both ω-hydroxylation and epoxidation of arachidonic acid by Sf9-expressed CYP4A isoforms are inhibited by 17-ODYA, a widely used acetylenic inhibitor of fatty acid metabolism (Zou et al., 1994c; Nguyen et al., 1999). 17-ODYA also potently inhibited the CYP4F1- and CYP4F4-mediated ω-hydroxylation of arachidonic acid with an IC₅₀ of 1.81 and 5.59 $\mu$M, respectively (Table 3 and Fig. 8A). DDMS, a specific inhibitor of microsomal arachidonic acid ω-hydroxylation, potently decreased ω-
and ω-1-hydroxylation and epoxidation catalyzed by CYP4A1, CYP4A2, and CYP4A3, with a similar IC₅₀ for all isoforms (Wang et al., 1998). In contrast, it had no effect on CYP4F1-mediated ω-hydroxylation of arachidonic acid and only slightly inhibited CYP4F4-mediated 20-HETE formation with an IC₅₀ of 145 μM (Table 3). 10-SUYS, a specific inhibitor of microsomal arachidonic acid ω-hydroxylation (Xu et al., 2002), inhibited the arachidonic acid ω-hydroxylase activity of CYP4F1 and CYP4F4 with an IC₅₀ of 231 and 25.0 μM, respectively (Table 3 and Fig. 8B). ABT has been characterized as a specific inhibitor of microsomal arachidonic acid ω-hydroxylation (Su et al., 1998), and it inhibited the CYP4F1- and CYP4F4-mediated ω-hydroxylation of arachidonic acid with an IC₅₀ of 289 and 371 μM, respectively (Table 3 and Fig. 8C). With the exception of ABT, CYP4F1 and CYP4F4 showed different sensitivity to the P450 hydroxylase inhibitors tested (Table 3 and Fig. 8).

**Discussion**

The emerging importance of 20-HETE in the regulation of vascular tone and renal tubular transport has led to a re-
newed interest in understanding the mechanisms that regulate 20-HETE formation within the kidney. The \( \omega \)-hydroxylation of arachidonic acid to form 20-HETE has generally been attributed to the CYP4A isoforms (Nguyen et al., 1999; Hoch et al., 2000). More recently, members of the CYP4F family have been cloned and characterized with respect to their catalytic function, tissue expression, and transcriptional regulation (Chen and Hardwick, 1993; Kawashima and Strobel, 1995; Kawashima et al., 1997; Kikuta et al., 1999; Cui et al., 2000, 2001; Zhang et al., 2000). Much of the interest in the CYP4F enzymes has focused on their role in the \( \omega \)-hydroxylation of LTB4, an important step in controlling the potent inflammatory properties of this eicosanoid (Sumi-moto et al., 1988). In contrast, little is known about the ability of the CYP4F proteins to catalyze arachidonic acid metabolism. Although the rat CYP4F isoforms have been heterologously expressed in both yeast and \( E. \ coli \) (Kawashima et al., 1997; Kikuta et al., 1999; Bylund et al., 2003), this represents the first study in which the catalytic function of all four rat isoforms has been directly compared. Despite more than 70% amino acid sequence identity, the rat CYP4F isoforms show differences in substrate specificity and sensitivity to known P450 inhibitors. Both human and rat CYP4Fs have been characterized as LTB4 \( \omega \)-hydroxylases (Kikuta et al., 1993, 1999; Kawashima et al., 1997). The current study suggests that the majority of the LTB4 \( \omega \)-hydroxylase activity in the rat is catalyzed by CYP4F1 and CYP4F4. The \( K_m \) for CYP4F1 in the present study (24 \( \mu \)M) is much lower than that reported for CYP4F1 expressed in yeast (134 \( \mu \)M; Kikuta et al., 1999), possibly due to the different expression systems and incubation conditions. The activity of CYP4F4 was also much higher than that reported previously for an \( E. \ coli \)-expressed cell lysate (Kawashima et al., 1997). Despite repeated attempts to optimize the reconstitution and incubation conditions for CYP4F5 and CYP4F6, metabolism of LTB4 was difficult to detect. Minor activity was detected with CYP4F5, whereas CYP4F6 was devoid of LTB4 \( \omega \)-hydroxylase activity. These results are in conflict with the extensive side-chain hydroxylation of LTB4 reported for yeast-expressed CYP4F5 and CYP4F6 (Bylund et al., 2003). Although CYP4F1 and CYP4F4 are clearly implicated in the metabolism of LTB4 and regulation of inflammation, further studies will be necessary to clarify the role of CYP4F5 and CYP4F6 in this pathway.

A direct comparison of the arachidonic acid \( \omega \)-hydroxylase activity of all four rat CYP4F isoforms identified CYP4F1 and CYP4F4 as major catalysts of 20-HETE formation. In fact, the \( k_{cat} \) values for CYP4F1 and CYP4F4 were very similar to those reported previously for the most active CYP4A isoform, CYP4A1 (Hoch et al., 2000). This is consistent with the identification of CYP4F2 as the major arachidonic acid \( \omega \)-hydroxylase in human liver (Lasker et al., 2000). However, the inability of yeast-expressed CYP4F4 (Kikuta et al., 1999) and \( E. \ coli \)-expressed CYP4F4 (Kawashima et al., 1997) to metabolize arachidonic or lauric acid highlights the importance of determining optimal conditions for reconstitution of P450 activity with expressed proteins. Although the
relative importance of the CYP4A and CYP4F isoforms in 20-HETE formation within rat tissues is still unclear, these results support a potential role for both enzyme families in this catalytic function.

A major difference in the activity of the CYP4A and CYP4F isoforms is reflected in the inability of most of the latter enzymes to metabolize lauric acid. CYP4A isoforms readily catalyze the \( \omega \)-hydroxylation of lauric acid, which is the prototypical substrate for this subfamily (Nguyen et al., 1999; Hoch et al., 2000). Elongation of the fatty acid chain is associated with a loss of CYP4A \( \omega \)-hydroxylase activity (Hoch et al., 2000). Although further studies will be necessary to determine the influence of hydrocarbon chain length on the activity of the CYP4F enzymes, these results establish distinct substrate specificity between the CYP4A and CYP4F subfamilies.

The identification of CYP4F1 and CYP4F4 as arachidonic acid \( \omega \)-hydroxylases and their detection in rat kidney (Kal-sotra et al., 2002) implicates them in the renal formation of 20-HETE. CYP4F1 accounts for 95% of total CYP4F expression in the Sprague-Dawley kidney, whereas CYP4F4 has the lowest expression (less than 0.01% of total CYP4F expression) (Kal-sotra et al., 2002). The abundant expression of CYP4F1 in the rat kidney and our finding that CYP4F1 has
similar arachidonic acid ω-hydroxylase activity to the most active CYP4A isoform, CYP4A1, suggests that CYP4F1 is the major CYP4F isoform that contributes to renal 20-HETE biosynthesis in the kidney. Relatively little is known about the regulation of the renal CYP4F enzymes. Clofibrate treatment decreased CYP4F1, CYP4F5, and CYP4F6 and had no effect on CYP4F4 mRNA levels in rat liver (Kalsotra et al., 2002). Although tissue-specific differences in response to fibric acids make it difficult to predict the effect of this classic inducer on renal CYP4F levels, it is interesting to note that in the liver, CYP4F1 is repressed, whereas CYP4A1 is induced by clofibrate. This suggests that the increased 20-HETE formation expected from CYP4A1 induction by clofibrate may be somewhat balanced by the decrease in 20-HETE formation due to CYP4F1 repression. Renal CYP4F expression was also reported to be higher in females relative to males and to be regulated by estrogen. This is in contrast to CYP4A expression, which is higher in males than females. This would support a differential role for the CYP4F and CYP4A isoforms in 20-HETE formation in males and females. Interestingly, Kalsotra et al. (2002) also reported that the expression level of lung CYP4F4 can be induced up to 10-fold 24 h after traumatic brain injury. Our results indicate that CYP4F4 has higher arachidonic acid ω-hydroxylase activity than CYP4F1, but its low expression in kidney does not support a major role for this isoform in renal 20-HETE formation. It will be interesting to examine whether CYP4F4 plays its major role in 20-HETE biosynthesis in other tissues during such conditions as traumatic brain injury.

The potent vasoconstrictor effect of 20-HETE on renal arteries is considered prohypertensive, whereas its natriuretic and diuretic effects in renal tubules would contribute to a lowering of blood pressure. The relative importance of the CYP4F and CYP4A isoforms to renal 20-HETE formation will be a function of their expression pattern within the renal tubules and microvessels as well as the catalytic function of each enzyme. CYP4A and CYP4F mRNAs are expressed in a segment- and gender-specific pattern in the mouse kidney (Stec et al., 2003). Characterization of the corresponding expression pattern in the rat kidney will provide important information about the relative contribution of each of the CYP4F and CYP4A isoforms to renal 20-HETE formation.

P450 inhibitors are an important tool in characterizing the biological significance of 20-HETE. CYP4F1 and CYP4F4 displayed different sensitivity to several inhibitors of P450 ω-hydroxylase activity. Surprisingly, DDMS, a very potent inhibitor of CYP4A-catalyzed arachidonic acid ω-hydroxylation and epoxidation (Nguyen et al., 1999), had minimal effect on CYP4F1- or CYP4F4-catalyzed 20-HETE formation. 10-SUYS, a specific inhibitor of microsomal arachidonic acid ω-hydroxylation (Xu et al., 2002), was a relatively potent inhibitor of CYP4F1 but had only a minor effect on CYP4F4. This suggests that 10-SUYS can be used at low concentrations to selectively inhibit CYP4F4 fatty acid ω-hydroxylase activity and that DDMS can be used to selectively inhibit CYP4A but not CYP4F arachidonic acid ω-hydroxylase activity. In contrast, 17-ODYA and ABT showed similar effects on CYP4F1 or CYP4F4, consistent with their relatively nonspecific inhibition of arachidonic acid hydroxylation and epoxidation (Wang et al., 1998; Nguyen et al., 1999; Alonso-Galicia et al., 2002). The isoform selectivity observed in this study is consistent with the incomplete inhibition of arachidonic acid ω-hydroxylation observed with many P450 inhibitors when used in microsomal systems. The identification of isoform specific inhibitors such as 10-SUYS and DDMS will be invaluable for characterizing the relative contribution of a given CYP4A and/or CYP4F enzyme to 20-HETE formation.

In summary, CYP4F1 and CYP4F4 have been identified as arachidonic acid ω-hydroxylases. In light of the abundant expression of CYP4F1 in the rat kidney (Kalsotra et al., 2002), CYP4F1 may be a major contributor to the biosynthesis of 20-HETE in the rat kidney. Characterization of isoform-selective inhibition of arachidonic acid ω-hydroxylation supports the development of novel P450 inhibitors for widespread use in the in vivo and in vitro characterization of the biological properties of 20-HETE.
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References


Address correspondence to: Dr. Deanna L. Kroetz, Department of Biopharmaceutics, College of Pharmacy, University of California, 513 Parnassus, Box 4446, San Francisco, CA 94143-0446. E-mail: deanna@itsa.ucsf.edu