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Immunochemical quantification of cynomolgus CYP2J2, CYP4A, and CYP4F enzymes in liver and small intestine

Shotaro Uehara^{a,b}, Norie Murayama^b, Yasuharu Nakanishi^a, Chika Nakamura^a, Takanori Hashizume^c, Darryl C. Zeldin^d, Hiroshi Yamazaki^{b,*}, and Yasuhiro Uno^{a,*}

^aPharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Kainan, Wakayama 642-0017, Japan

^bLaboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan

^cFaculty of Pharmacy, Osaka Ohtani University, Tondabayashi, Osaka, Japan

^dDivision of Intramural Research, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina, U.S.A

Abstract

1. An increasing number of studies have indicated the roles of CYP4 proteins in drug metabolism; however, CYP4 expression has not been measured in cynomolgus monkeys, an important animal species for drug metabolism studies.
2. In this study, cynomolgus CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, along with CYP2J2, were immunoquantified using selective antibodies in 28 livers and 35 small intestines, and their content was compared with CYP1A, CYP2A, CYP2B6, CYP2C9/19, CYP2D, CYP2E1, CYP3A4, and CYP3A5, previously quantified.
3. In livers, CYP2J2, CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, varied 1.3- to 4.3-fold, represented 11.2%, 14.4%, 8.0%, 2.7%, and 0.3% of total immunoquantified CYP1-4 proteins, respectively.
4. In small intestines, CYP2J2, CYP4F2/3, CYP4F11, and CYP4F12, varied 2.4- to 9.7-fold, represented 6.9%, 36.4%, 2.4%, and 9.3% of total immunoquantified CYP1-4 proteins, respectively, making CYP4F the most abundant P450 subfamily in small intestines. CYP4A11 was under the detection limit in all of the samples analyzed.
5. Significant correlations were found in liver for CYP4A11 with lauric acid 11-/12-hydroxylation and for CYP4F2/3 and CYP4F11 with astemizole hydroxylation.
6. This study revealed the relatively abundant contents of cynomolgus CYP2J2, CYP4A11, and CYP4Fs in liver and/or small intestine, suggesting their potential roles for the metabolism of xenobiotics and endogenous substrates.

*Corresponding authors: Yasuhiro Uno, D.V.M., Ph.D. Genome Research Group, Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., 16-1 Minami Akasaka, Kainan 642-0017, Japan. Phone: +81-73-483-8881. Fax: +81-73-483-7377. uno-yasuhiro@snbl.co.jp Or Hiroshi Yamazaki, Ph.D. Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3-3165 Higashi-Tamagawa Gakuen, Machida, Tokyo 194-8543, Japan. Phone: +81-42-721-1406. Fax: +81-42-721-1406. hyamazak@ac.shoyaku.ac.jp.

Keywords

Cynomolgus monkey; cytochrome P450; expression; liver; small intestine

Introduction

Cytochromes P450 (P450) is gene superfamily consisting of a large and diverse group of heme proteins involved in the metabolism of numerous xenobiotics and endogenous substrates. In human P450s, 57 functional genes and 58 pseudogenes have been identified (Nelson et al. 2004). In general, CYP1-3 enzymes play major roles for drug metabolism, while CYP4 enzymes, including CYP4A11, CYP4F2, CYP4F3B, CYP4F11, and CYP4F12, are involved mainly in metabolism of endogenous compounds such as eicosanoids (*e.g.* arachidonic acid, leukotriene B₄, and prostaglandins) (Kalsotra and Strobel 2006; Hsu et al. 2007). CYP4 enzymes are also involved in metabolism of several drugs. Human CYP4F2 and CYP4F3B are involved in metabolism of the antiparasitic prodrug pafuramidine (DB289), and CYP4F enzymes are responsible for metabolism of ebastine (H1-antihistamine prodrug) in small intestine (Hashizume et al. 2001; Hashizume et al. 2002). Similarly, CYP2J2 also participates in metabolism of drugs such as astemizole (Matsumoto et al. 2002) and ebastine (Hashizume et al. 2002) in addition to endogenous substrates such as arachidonic acid.

The cynomolgus monkey (*Macaca fascicularis*) is a widely used primate species in drug development. In cynomolgus monkeys, more than 23 P450 enzymes, highly homologous to human P450s, have been identified, including CYP1-4 enzymes (Uno et al. 2011a). Five CYP4 enzymes have been identified in cynomolgus monkeys, including CYP4A11, CYP4F2, CYP4F3v2, CYP4F11, and CYP4F12 (Uno et al. 2011a). The genes for all these enzymes are expressed in liver, while only *CYP4F2*, *CYP4F11*, and *CYP4F12* are expressed in small intestine (Uno et al. 2007). In our previous study, the immunoquantification of cynomolgus CYP1-3 enzymes in 28 livers revealed that CYP3A was the most abundant subfamily as in human liver (Shimada et al. 1994), followed by CYP2A, CYP2E1, CYP2B6, CYP2C9/19, CYP2C76, and CYP2D (Uehara et al. 2011). Similarly, the immunoquantification of cynomolgus CYP1-3 enzymes in 35 small intestines revealed that CYP3A was the most abundant subfamily as in human small intestine (Paine et al. 2006), followed by CYP2J2, CYP2C9/19, CYP1A, and CYP2D (Uehara et al. 2014).

A recent report indicated the metabolism of human CYP4F substrates in cynomolgus monkey small intestines (Nishimuta et al. 2011). Moreover, cynomolgus CYP4F is involved in metabolism of ebastine in small intestines (Hashizume et al. 2001), indicating the potential importance of CYP4F enzymes for metabolism of some drugs in cynomolgus monkeys. Importantly, protein expression and function of cynomolgus CYP4F enzymes have not been fully investigated in liver or small intestine. In this study, therefore, expression of cynomolgus CYP4A and CYP4F enzymes, along with CYP2J2, was immunoquantified using selective antibodies in 28 livers and 35 small intestines.

Materials and Methods

Chemicals and reagents

Anti-human CYP2J2 antibodies used for immunoblot analysis were prepared as described previously (King et al. 2002). Anti-rat CYP4A antibodies and anti-human CYP4F11 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-human CYP4F3 antibodies were purchased from Abnova (Taipei, Taiwan). Anti-human CYP4F12 antibodies were purchased from Abcam (Cambridge, MA). The secondary antibodies (goat anti-mouse, donkey anti-goat, and goat anti-rabbit horseradish peroxidase-conjugated IgGs) were purchased from Santa Cruz Biotechnology. Polyvinylidene difluoride (PVDF) membranes and reagents for enhanced chemiluminescence were purchased from GE Healthcare (Piscataway, NJ). [$1\text{-}^{14}\text{C}$] Lauric acid was purchased from American Radiolabeled Chemicals (St. Louis, MO). All other chemicals and reagents were of electrophoresis or analytical grade where appropriate.

Preparation of microsomes and recombinant P450 proteins

Liver and small intestine (jejunum) samples were collected from 28 cynomolgus monkeys (14 males and 14 females from Indochina or Indonesia) and 35 cynomolgus monkeys (17 males and 18 females from Cambodia), respectively. This study was reviewed and approved by Institutional Animal Care and Use Committee at Shin Nippon Biomedical Laboratories, Ltd. Liver and small intestine microsomes were prepared as described previously (Uehara et al. 2011; Uehara et al. 2014). Concentration of total proteins was determined by the Bradford method using Bio-Rad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Five recombinant cynomolgus P450 proteins (CYP2J2, CYP4A11, CYP4F2, CYP4F11, and CYP4F12) were expressed in *Escherichia coli*, and membrane preparation was performed as described previously (Uno et al. 2011b). The content of each P450 protein in the membrane preparation was determined by $\text{Fe}^{2+} \cdot \text{CO}$ vs. Fe^{2+} difference spectra as described previously (Omura and Sato 1964).

Immunoblotting

Immunoblotting was performed as described previously (Uehara et al. 2011) using anti-human CYP2J2 antibodies, anti-rat CYP4A antibodies, anti-human CYP4F3 antibodies, anti-human CYP4F11 antibodies. The specificity of each antibodies was assessed using 24 recombinant P450 proteins (0.10-1.0 pmol), including cynomolgus CYP1A1, CYP1A2, CYP1D1, CYP2A23, CYP2A24, CYP2A26, CYP2B6, CYP2C8 (formerly known as CYP2C20), CYP2C9 (formerly known as CYP2C43), CYP2C18, CYP2C19 (formerly known as CYP2C75), CYP2C76, CYP2D17, CYP2D44, CYP2E1, CYP2J2, CYP3A4, CYP3A5, CYP3A43, CYP4A11, CYP4F2, CYP4F3(v2), CYP4F11, and CYP4F12. To measure expression of P450 proteins in 28 livers and 35 small intestines of cynomolgus monkeys, microsomal samples (10-30 μg) were electrophoresed and transferred to the PVDF membranes, which were subsequently incubated at room temperature with the primary antibodies (1:1000 to 1:5000 dilution) for 1 h, and later with the secondary antibodies (1:5000 dilution) for 30 min. For quantification of each P450 in microsomes, standard curves were generated using the recombinant protein of cynomolgus CYP4A11, CYP4F2, CYP4F11, and CYP4F12 for detection of cynomolgus CYP4A11, CYP4F2/3, CYP4F11,

and CYP4F12 in microsomes, respectively. Specific content was determined as described previously (Uehara et al. 2011).

Measurement of drug-metabolizing activity

Enzyme activity of CYP4F was measured using astemizole and ebastine as described previously (Yamazaki et al. 2006). Briefly, astemizole or ebastine (20 μ M) was incubated in 50 mM potassium phosphate buffer (pH 7.4) with liver microsomes of cynomolgus monkey and an NADPH-generating system (0.25 mM NADP⁺, 2.5 mM glucose 6-phosphate, and 0.25 unit/ml glucose 6-phosphate dehydrogenase) in a total volume of 0.20 ml. Reactions were incubated at 37°C for 30 min and terminated by addition of acetonitrile. After centrifugation (2800 g, 10 min), the supernatant was evaporated to dryness, and the residue was dissolved with methanol. Samples were analyzed by high-performance liquid chromatography with a C18 5- μ m analytical column. The mobile phase consisted of 6 mM ammonium acetate buffer (pH 4.5) and 40% acetonitrile at a flow rate of 1.0 ml/min for astemizole analyses. For ebastine analyses, the proportion of acetonitrile was maintained at 48% from 0 to 8 min and then increased linearly to 100% at 15 min. The elution profiles of the metabolites from ebastine and astemizole were monitored by ultraviolet light detection at 256 and 250 nm, respectively.

For lauric acid hydroxylation, 100 mM phosphate buffer containing 0.1 mM EDTA (pH 7.4) and ¹⁴C-labeled lauric acid (100 μ M) were used. The reaction was initiated by addition of an NADPH-generating system (1.25 mM NADP⁺, 3.3 mM glucose 6-phosphate, and 0.4 unit/ml glucose 6-phosphate dehydrogenase) in a final volume of 0.50 ml, and incubated at 37°C for 60 min. The incubation was carried out in a linear range of protein concentration and incubation time. The reaction was terminated by adding 0.25 ml of ice-cold acetonitrile/acetic acid (94/6, v/v). The mixtures were centrifuged at 7500 g for 10 min at 4°C and the metabolites in the supernatant were analyzed by radio high-performance liquid chromatography.

Results

Specificity of the antibodies for immunoblotting

To assess the specificity of anti-rat CYP4A antibodies, anti-human CYP4F3 antibodies, anti-human CYP4F11 antibodies, and anti-human CYP4F12 antibodies, immunoblotting was performed using recombinant proteins of 24 cynomolgus recombinant P450 proteins (Fig. 1). CYP4A11, CYP4F11, and CYP4F12 were selectively detected by anti-rat CYP4A, anti-human CYP4F11, and anti-human CYP4F12, respectively. Anti-human CYP4F3 antibody reacted with both CYP4F2 and CYP4F3, but not with any other P450s. Therefore, anti-rat CYP4A, anti-human CYP4F3 antibody, anti-human CYP4F11, and anti-human CYP4F12 were subsequently used for quantification of cynomolgus CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, respectively. Specificity of anti-human CYP2J2 antibodies was previously confirmed (Uehara et al. 2011).

Quantification in 28 cynomolgus monkey livers

To measure the expression of CYP2J2, CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12 in 28 cynomolgus monkey livers, immunoblotting was performed using the selective P450 antibodies. We previously reported that CYP2J2 protein in cynomolgus monkey liver was below the detection limit (Uehara et al. 2011) because the size of the bands detected with anti-CYP2J2 antibodies were different from that of recombinant CYP2J2 protein. However, we later noted that the size of human CYP2J2 protein bands appear different from that of recombinant CYP2J2 protein bands (Wu et al. 1996), cynomolgus CYP2J2 was re-analyzed in this study.

The standards and the representative five liver samples are shown in Fig. 2A. CYP2J2, CYP4A11, and CYP4F12 were detected in all 28 liver samples, while CYP4F2/3 and CYP4F11 were under the detection limit in 12 and 8 animals, respectively. Among CYP2J2, CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, CYP4A11 was most abundant (24.5 pmol/mg protein), ranging from 10.0 to 43.1 in different monkeys (Table 1). This was followed by CYP2J2, CYP4F2/3, CYP4F11, and CYP4F12, which averaged 22.1, 14.7, 4.2, and 0.6 pmol/mg protein, averaging from 12.5 to 33.8, 12.8 to 16.3, 2.6 to 5.2, and 0.3 to 0.9 pmol/mg protein, respectively, in different monkeys. These values were comparable to those of the pooled samples (Table 1). Depending on P450 enzyme, the content varied 1.3-fold (CYP4F2/3) to 4.3-fold (CYP4A11) among the animals analyzed (Fig. 3). No sex differences were observed in any P450 content.

By comparing the data of the pooled samples to the hepatic contents of CYP1A, CYP2A, CYP2B6, CYP2C9/19, CYP2D, CYP2E1, CYP3A4, and CYP3A5 which were previously immunoquantified in the same 28 samples (Uehara et al. 2011), CYP3A (3A4+3A5) was the most abundant subfamily (22.6% of total immunoquantified CYP1-4 proteins), followed by CYP2A (15.7%), CYP4A (14.4%), CYP2J (11.2%), CYP4F (11.0%), CYP2C (2C9/19+2C76) (9.4%), CYP2B (8.3%), CYP2E (7.2%), and CYP2D (0.2%) (Fig. 4A). The content of each P450, expressed as a percentage of total immunoquantified CYP1-4 proteins, ranged from 6.4 to 27.6, 8.2 to 14.1, 1.7 to 5.0, and 0.2 to 0.6% for CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, respectively. These results indicated that CYP3A was the most abundant subfamily in liver, but CYP2J2, CYP4A, and CYP4F were also abundantly expressed.

Quantification in 35 cynomolgus monkey small intestines

Similarly, expression of CYP4A11, CYP4F2/3, CYP4F11 and CYP4F12 were measured in 35 cynomolgus monkey small intestines by immunoblotting using the same antibodies. The results of the standards and the five representative small intestine samples are shown in Fig. 2B. CYP4F2/3, CYP4F11, and CYP4F12 were detected in all 35 animals, whereas CYP4A11 was under the detection limit (3.3 pmol/mg protein) in all of the 35 samples analyzed. Among CYP4F2/3, CYP4F11, and CYP4F12, CYP4F2/3 was the most abundant (16.0 pmol/mg protein), ranging from 9.4 to 22.5 pmol/mg protein (Table 2). This was followed by CYP4F12 and CYP4F11, which averaged 5.0 and 1.0 pmol/mg protein, ranging from 2.4 to 8.2 and 0.3 to 2.9 pmol/mg protein, respectively. These values were similar to those of the pooled samples (Table 1). Each P450 content varied 2.4-fold (CYP4F2/3) to

9.7-fold (CYP4F11) among the animals analyzed (Fig. 3), depending on P450 enzyme. No sex differences were observed in any P450 content.

By comparing the data of the pooled samples to the contents of CYP1A, CYP2C9/19, CYP2D, CYP2J2, CYP3A4, and CYP3A5 which were previously immunoquantified in the same 35 small intestine samples (Uehara et al. 2014), CYP4F (CYP4F2/3+CYP4F11+CYP4F12) was the most abundant subfamily (48.1% of total immunoquantified CYP1-4 proteins), followed by CYP3A (CYP3A4+CYP3A5) (41.2%), CYP2J (6.9%), CYP2C(9/19) (2.0%), and CYP2D (0.2%) (Fig. 4B). The content of each P450, expressed as a percentage of total immunoquantified CYP1-4 proteins, ranged from 20 to 42, 0.6 to 5.9, 5.4 to 14% for CYP4F2/3, CYP4F11, and CYP4F12, respectively. These results indicated that CYP4F was the most abundant subfamily with the content higher than CYP3A in cynomolgus monkey small intestine.

Correlation to drug-metabolizing enzyme activities

To correlate with each P450 content, drug-metabolizing enzyme activities were measured in 28 liver microsomes using astemizole, ebastine, and lauric acid as substrate. The enzyme activity in the small intestine microsomes was not measured because quantity of the samples was not sufficient for the experiment. Correlation analysis indicated that CYP4A11 was significantly correlated with lauric acid 11- and 12-hydroxylation (Table 3). CYP4F2/3 and CYP4F11 were also significantly correlated with astemizole hydroxylation. In contrast, a significant correlation was not observed for astemizole *O*-demethylation or ebastine hydroxylation.

Discussion

To quantify cynomolgus CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12 content, immunoblotting was performed using selective antibodies in 28 liver microsomes and 35 small intestine microsomes. CYP2J2 was also analyzed in the 28 liver microsomes. Cynomolgus CYP4A was the third abundant subfamily in liver, representing 14.4% of total immunoquantified CYP1-4 proteins (Fig. 4). Cynomolgus *CYP4A11* is predominantly expressed in liver and kidney, and the enzyme metabolizes arachidonic acid (Uno et al. 2011b), similar to human *CYP4A11* (Hsu et al. 2007). CYP2J was the fourth abundant subfamily in liver, representing 11.2% of total immunoquantified CYP1-4 proteins (Fig. 4). In human, mean CYP2J2 content was 2.0 and 1.2 pmol/mg protein, representing 1.8 and 0.52% of total hepatic P450 content in Japanese and Caucasians, respectively (Yamazaki et al. 2006). Higher content of cynomolgus CYP2J2 (18.2 pmol/mg protein) might partly account for the higher hepatic CL_{int} value of astemizole in cynomolgus monkeys than in humans (Nishimuta et al. 2011).

In cynomolgus monkey livers, CYP4F (CYP4F2/3+CYP4F11+CYP4F12) was the fifth abundant subfamily, representing 11% of total immunoquantified CYP1-4 proteins, which is a sum of CYP4F2/3 (8%), CYP4F11 (2.7%), and CYP4F12 (0.3%). A recent study showed that CYP4F (CYP4F2 +CYP4F3B) is the fourth abundant subfamily in human livers, representing 15% of total P450 content determined by LC-MS/MS (Michaels and Wang 2014). Human CYP4F content (CYP4F2 +CYP4F3B, 25.5 pmol/mg protein) in that study is

more abundant than cynomolgus CYP4F content (CYP4F2/3+CYP4F11+CYP4F12, 19.5 pmol/mg protein) determined in this study (Table 1). Similarly, human CYP4F11 content (8.4 pmol/mg protein) (Edson et al. 2013) is more abundant than cynomolgus CYP4F11 content (4.2 pmol/mg protein) determined in this study (Table 1). These results indicated that overall CYP4F content of cynomolgus monkey livers is less than that of human livers.

In cynomolgus monkey small intestines, CYP4F (CYP4F2/3+CYP4F11+CYP4F12) was the most abundant P450 subfamily, representing 48.1% of total immunoquantified CYP1-4 proteins: CYP4F2/3, CYP4F11, and CYP4F12 content represent 36.4%, 2.4%, and 9.3%, respectively (Fig. 4B). Cynomolgus CYP4F content was higher in small intestines (22.0 pmol/mg protein) (Table 2) than in liver (19.5 pmol/mg protein) (Table 1). *CYP4F2* is abundantly expressed, but *CYP4F3* is expressed marginally in cynomolgus small intestine (Uno et al. 2007), suggesting that CYP4F2/3 protein expression largely consisted of CYP4F2. In small intestines, cynomolgus CYP4F enzymes are involved in metabolism of ebastine (Hashizume et al. 2001), and another study suggested the involvement of CYP4F enzymes in metabolism of astemizole and terfenadine (Nishimuta et al. 2011). CYP4F content was larger in cynomolgus monkey small intestines (22.0 pmol/mg protein) (Table 2) than in human small intestines (7 pmol/mg protein) (Wang et al. 2007). Moreover, actual CYP4F content might be higher because P450 content immunoquantified using unpurified recombinant enzymes as standards might be lower than the actual content (Perrett et al. 2007). An increasing number of drugs are being found that are metabolized by CYP4Fs (Kalsotra and Strobel 2006), including pafuramidine (antiparasitic prodrug) (Wang et al. 2007) and fingolimod (for treatment of multiple sclerosis) (Jin et al. 2011). These observations suggest the potential importance of cynomolgus CYP4F enzymes for first-pass metabolism in the small intestine.

Human CYP4F2 is a vitamin K1 oxidase in the liver, and carriers of the variant allele (V433M) have a reduced capacity to metabolize vitamin K (McDonald et al. 2009). Patients carrying the *CYP4F2* V433M allele are likely to have elevated hepatic levels of vitamin K, which require a higher dose of warfarin to achieve a therapeutic anticoagulant response. This *CYP4F2* allele, along with the *CYP2C9* and *VKORC1* alleles, partly accounts for inter-individual variability in response to warfarin, but contribution of each allele appears to depend on ethnicity (Takeuchi et al. 2009; Danese et al. 2012; Nakamura et al. 2012). Because cynomolgus P450 genes are also polymorphic (Uno et al. 2009; Uno et al. 2010; Uno and Osada 2011), similar variants in cynomolgus *CYP4F2* might account for inter-animal variations of CYP4F2-mediated drug metabolism.

The variation of each CYP4F content was relatively small in the cynomolgus monkey livers (1.3- to 3.0-fold) (Table 1). The previous study showed that in human livers, CYP4F2 content varied 3.4-fold when two donors showing the lowest expression were excluded from the analysis, and CYP4F3B content varied 3.3-fold (Michaels and Wang 2014). In small intestines, the variation of CYP4F2/3 and CYP4F12 content was also relatively small (2.4- and 3.4-fold, respectively), but CYP4F11 content varied more (9.7-fold) (Table 2). The individual variation of human CYP4F content in small intestines is relatively small (6-fold) (Wang et al. 2007). These results indicated that individual variations of CYP4F content in liver and small intestine are generally similar between cynomolgus monkeys and humans.

The correlation analysis between P450 content and hepatic enzyme activities revealed significant correlation for CYP4A11 with lauric acid 11-hydroxylation and lauric acid 12-hydroxylation (Table 3), raising the possibility that lauric acid is metabolized by cynomolgus CYP4A11, similar to human CYP4A11 (Hsu et al. 2007). CYP4F2/3 and CYP4F11 were significantly correlated with astemizole hydroxylation (Table 3). A previous study reported the potential involvement of cynomolgus CYP4F enzymes in metabolism of astemizole in small intestine (Nishimuta et al. 2011), possibly accounted for by CYP4F2/3 and CYP4F11, which were expressed abundantly in small intestine (Fig. 4B). Interestingly, CYP4F12 content was not significantly correlated with any of the reactions analyzed, including ebastine hydroxylation (Table 3), which is catalyzed by cynomolgus CYP4F12 (Hashizume et al. 2001). This discrepancy might be due to the larger contribution of CYP4F12 to ebastine metabolism in small intestine than in liver, similar to humans (Hashizume et al. 2001). Moreover, other cynomolgus P450s are involved in ebastine metabolism, considering that ebastine hydroxylation is also catalyzed by not only CYP4F12, but also CYP2J2, CYP3A4, CYP3A5, and CYP4F2 in humans (Hashizume et al. 2002; Liu et al. 2006). Although the limited quantity of the remaining microsome samples prevented us from analyzing enzyme assays in the small intestines, such investigation would be helpful to determine correlation of each CYP4F content to drug-metabolizing enzyme activities in cynomolgus monkey small intestines.

In conclusion, immunochemical quantification of cynomolgus CYP2J2, CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12, together with cynomolgus CYP1A, CYP2A, CYP2B6, CYP2C9/19, CYP2C76, CYP2D, CYP2E1, CYP3A4, and CYP3A5 content, revealed that CYP4F (CYP4F2/3, CYP4F11, and CYP4F12) was the most abundant subfamily in small intestines, representing 48.1% of total immunoquantified CYP1-4 proteins, followed by CYP3A (41.2%), CYP2J2 (6.9%), CYP2C9/19 (2.0%), CYP1A (1.6%), and CYP2D (0.2%). In cynomolgus monkey livers, CYP3A was the most abundant subfamily, representing 22.6% of total immunoquantified CYP1-4 proteins, followed by CYP2A (15.7%), CYP4A (14.4%), CYP2J2 (11.2%), CYP4F (11%), CYP2C (9.4%), CYP2B (8.3%), CYP2E (7.2%), and CYP2D (0.2%). Individual variations were relatively small in livers (CYP2J2, CYP4A11, CYP4F2/3, CYP4F11, and CYP4F12) and small intestines (CYP4F2/3 and CYP4F12), similar to humans. This study suggested potential important roles of CYP4F enzymes for drug metabolism in cynomolgus monkeys, and helps to further understand the similarities and differences of a P450-dependent drug metabolism between cynomolgus macaques and humans.

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Figure 1.

Immunoblots demonstrating specificity of P450 antibodies. To determine the specificity of P450 antibodies, 24 cynomolgus recombinant P450 proteins (0.10 pmol of P450/lane) were immunoblotted using anti-rat (r) CYP4A antibodies, anti-human (h) CYP4F11 antibodies, and anti-human CYP4F12 antibodies. For anti-human CYP4F3 antibodies, 1 pmol of P450/lane was used. Anti-rat CYP4A antibodies, anti-human CYP4F3 antibodies, anti-human CYP4F11 antibodies, and anti-human CYP4F12 antibodies were specifically reacted with cynomolgus CYP4A11 (59 kDa), CYP4F2/3 (60 kDa), CYP4F11 (60 kDa), and CYP4F12 (60 kDa), respectively.

A **B** **Figure 2.**

Immunoblotting of cynomolgus monkey liver and small intestine microsomes. The amounts of standards (cynomolgus recombinant P450 proteins) ranged from 0.010 to 0.10 (CYP2J2), 0.025 to 0.125 (CYP4A11), 0.025 to 0.30 (CYP4F2), 0.025 to 0.15 (CYP4F11), and 0.01 to 0.10 pmol (CYP4F12). The results of five animals are shown as representative examples. (A) Immunoblotting was performed using liver microsomes; 5 µg for CYP4A11, 10 µg for CYP2J2 and CYP4F11, or 20 µg for CYP4F2/3 and CYP4F12. The pooled sample was prepared from 14 male and 14 female microsomes. (B) Immunoblotting was performed using small intestine microsomes; 10 µg for CYP4F11, 20 µg for CYP4A11 and CYP4F12, or 30 µg for CYP4F2/3. The pooled sample was prepared from 17 male and 18 female microsomes.

Figure 3.

Variations of P450 content in cynomolgus monkeys. (A) The contents of cynomolgus CYP4F2/3 and CYP4A11 in 28 livers. Among the 28 liver samples, the variation of CYP4F2/3 contents was the least (1.3-fold), and that of CYP4A11 contents was the most (4.3-fold). (B) The contents of cynomolgus CYP4F2/3 and CYP4F11 in 35 small intestines. Among the 35 small intestine samples, the variation of CYP4F2/3 contents was the least (2.4-fold), and that of CYP4F11 contents was the most (9.7-fold).

Figure 4.
P450 pie of cynomolgus monkey liver and small intestine. Mean expression value of each P450 content is expressed as percentage of the total immunoquantified CYP1-4 proteins for liver (A) and small intestine (B).

Table 1

Individual P450 content immunoquantified in 28 cynomolgus monkey livers.

P450	P450 content (Mean ± S.D.)				n	Range	Detection limit
	Pooled	Individuals	Males	Females			
		pmol/mg protein					
CYP2J2	18.2 ± 0.6	22.1 ± 4.4	20.6 ± 3.8	23.6 ± 4.5	28	12.5 – 33.8	2.0
CYP4A11	23.3 ± 1.0	24.5 ± 10.5	26.1 ± 9.0	23.0 ± 11.8	28	10.0 – 43.1	10.0
CYP4F2/3	12.9 ± 1.1	14.7 ± 1.0	15.4 ± 2.0	14.3 ± 0.4	16	12.8 – 16.3	12.5
CYP4F11	4.4 ± 0.4	4.2 ± 0.7	4.5 ± 1.4	4.1 ± 0.6	20	2.6 – 5.2	2.5
CYP4F12	0.5 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	28	0.3 – 0.9	0.3

Pooled microsomes were prepared from 14 male and 14 female microsomes.

Table 2
Individual P450 content immunoquantified in 35 cynomolgus monkey small intestines.

P450	P450 content (Mean ± S.D.)				n	Range	Detection limit
	Pooled	Individuals	Males	Females			
			pmol/mg protein				
CYP4F2/3	18.4 ± 1.7	16.0 ± 3.4	15.2 ± 3.2	16.8 ± 3.5	35	9.4 – 22.5	8.3
CYP4F11	1.2 ± 0.03	1.0 ± 0.7	0.9 ± 0.5	1.0 ± 0.8	35	0.3 – 2.9	0.25
CYP4F12	4.7 ± 0.4	5.0 ± 1.5	4.9 ± 1.3	5.2 ± 1.7	35	2.4 – 8.2	1.25

Pooled microsomes were prepared from 17 male and 18 female microsomes.

Table 3

Correlation coefficients (r) between P450 content and drug-metabolizing enzyme activities in 28 cynomolgus monkey livers.

Activity	2J2	4A11	4F2/3	4F11	4F12
<i>nmol/min/mg protein</i>					
Astemizole <i>O</i> -demethylation	0.34	0.34	0.03	0.11	-0.01
Astemizole hydroxylation	-0.15	0.37	0.38*	0.40*	0.12
Ebastine hydroxylation	-0.12	0.36	0.26	0.03	0.19
Lauric acid 12-hydroxylation	0.03	0.83*	0.12	0.35	0.38*
Lauric acid 11-hydroxylation	-0.29	0.58*	0.17	0.13	-0.04

Metabolic activities were measured as described in *Materials and Methods*. Values are shown as mean $\pm$ S.D. for 28 cynomolgus monkeys. Statistical significance was determined based on the P value (probability that r is zero) of the linear regression:

* $P < 0.05$.